

Nutrition Reviews

Volume 33

January 1975

Number 1

Active Vitamin D-Like Substances in Solanum malacoxylon and Other Calcinogenic Plants R. H. Wasserman	1
--	---

Clinical Nutrition

Growth of the Human Brain	6
Infant Body Composition by Skinfold Measurements	7
Folate Binder in Leukocytes and Serum ..	9
Serum Ferritin and Body Iron Stores ...	11

Nutrition Classics

Edith G. Willcock and F. Gowland Hopkins: "The Importance of Indi- vidual Amino-Acids in Metabolism" ..	15
---	----

Experimental Nutrition

Zinc Availability in Leavened and Un- leavened Bread	18
Nitrosamines and Cancer	19
Changes in Deficiency of Vitamin B ₆ and Other B-Complex Vitamins	21
Competition Between Phlorizin and Gold Thioglucose in Glucoreceptor Cells	23
Vitamin K and the Carboxylation of Glutamyl Residues	25

Nutrition Notes

Termination of AMA Council	29
Meeting Announcements	30
FTC Proposal on Food Advertising	30
Letter to the Editor	31
Recent Books	31

January 1975

Editor

D. M. Hegsted, Ph.D., Professor of Nutrition
Schools of Medicine and Public Health
Harvard University, Boston

Associate Editor

Elmer H. Stotz, Ph.D.
University of Rochester

Assistant Editor

Robin Holab

Contributing Editors

G. A. O. Alleyne, M.D. University of the West Indies	Olaf Mickelsen, Ph.D. Michigan State University
Eric G. Ball, Ph.D. Marine Biological Laboratory, Woods Hole	R. D. G. Milner, M.D., Ph.D. University of Manchester
Mahtab S. Bamji, Ph.D. National Institute of Nutrition, India	Robert A. Neal, Ph.D. Vanderbilt University
Robert G. Campbell, M.D. University of Rochester	Ralph A. Nelson, M.D., Ph.D. The Mayo Clinic
Richard M. Forbes, Ph.D. University of Illinois	Susan Oace, Ph.D. University of California
Charles H. Halsted, M.D. University of California	Mildred S. Rodriguez, Ph.D. Los Angeles, California
Frank L. Iber, M.D. University of Maryland	James I. Shaw, Ph.D. Harvard School of Dental Medicine
Kamala S. Jaya Rao, M.D. National Institute of Nutrition, India	D. A. T. Southgate, Ph.D. University of Cambridge
Michael C. Latham, M.B. Cornell University	Steven R. Tannenbaum, Ph.D. Massachusetts Institute of Technology
Gilbert A. Leveille, Ph.D. Michigan State University	Carol I. Waslien, Ph.D. Auburn University
Richard L. Lyman, Ph.D. University of California	Mary Ann Williams, Ph.D. University of California
Donald B. McCormick, Ph.D. Cornell University	Benjamin J. Wilson, Ph.D. Vanderbilt University
Robert B. McGandy, M.D. Harvard School of Public Health	Myron Winick, M.D. Columbia University

Editorial Office: 665 Huntington Avenue, Boston, Massachusetts 02115.

Send subscriptions and change of address requests to The Nutrition Foundation, Inc., 489 Fifth Avenue, New York, N.Y. 10017.

Subscription Rates: \$12.00 per year in the United States and other countries. A student rate of \$6.00 is offered to regularly matriculated students in colleges or universities.

Published by The Nutrition Foundation, Inc., 489 Fifth Avenue, New York, N.Y. 10017.

Second class postage paid at New York, N.Y. 10001 and at additional mailing offices.

© 1975 The Nutrition Foundation, Inc.

Note: Items may be quoted from NUTRITION REVIEWS without special permission. If quotations are made, the editors ask only that credit be given to NUTRITION REVIEWS and that they be made in such a way that the original meaning of the review is preserved.

Nutrition Classics: The editors extend their thanks to the authors and publishers of the original papers for their permission to reproduce these excerpts.

Active Vitamin D-Like Substances in *Solanum malacoxylon* and Other Calcinogenic Plants

by R. H. Wasserman, Ph.D.

"**E**nteque seco" is a disease of grazing animals that occurs in Argentina. The cause of this disease is due to the ingestion of the plant, *Solanum malacoxylon*, when the animals graze on contaminated pasture. This was clearly indicated from controlled studies in which the administration of the plant, or an extract thereof, duplicated the main features of the disease in different animal species.^{1,2} The symptoms and signs in the affected animals are, as follows: loss of weight, stiffness of forelimbs, arching of back, emaciation, and possibly death.¹ Autopsy reveals extensive soft tissue calcification, particularly of the cardiovascular system, tendons, ligaments, lungs, diaphragm, and kidney, with the extent of calcification varying with severity of the disease. These animals are usually hypercalcemic and hyperphosphatemic, such that the ion product of $Ca \times P$ in blood is considerably higher than normal.

Dr. Wasserman is Professor of Physical Biology at the New York State Veterinary College, Cornell University, Ithaca, New York 14853.

A limited number of reprints of this article may be obtained from the author. THERE ARE NO REPRINTS OF UNSIGNED REVIEWS.

In Brazil, the disease "Espichamento" was described by J. Döbereiner et al.³ The symptoms are quite similar to that of "Enteque seco" and the cause was also shown to be due to the ingestion of *Solanum malacoxylon*. Thus, "Enteque seco" and "Espichamento" appear to be equivalent diseases.

There have been described calcinotic diseases in grazing animals in other parts of the world. In the Alpine region of Germany, a disease of cattle and other herbivores, simply termed "enzootic calcosinosis", has been described by G. Dirksen and collaborators,⁴⁻⁶ and the clinical, histological, and biochemical signs and symptoms are similar to "Enteque seco". It has been clearly shown that this disease is produced by the ingestion of another plant, *Trisetum flavescens* (common name: oat-grass).⁶

Calcosinosis of grazing animals in still other regions are the "Manchester wasting disease"^{7,8} occurring in Jamaica and "Naahelu disease" in Hawaii.⁹ The former has not yet been related to any botanical species, but *Solanum sodomaeum* has been mentioned in connection with the disease in Hawaii.¹⁰ However, this author is not aware of investigations that conclusively demonstrate that *S. sodomaeum* is the toxic plant in this case.

Another botanical species that was recently implicated in causing calcinosis is *Cestrum diurnum*. This plant grows in the southeastern part of the United States, Jamaica, and Hawaii, and is now associated with calcinosis occurring in horses and cattle in the Miami area.^{11,12}

Thus, of the three plant species apparently responsible for calcinosis in animals, two are in the family *Solanaceae* (*S. malacoxylon* and *C. diurnum*), and one of the family *Gramineae* (*T. flavescens*).

Physiological studies showed that *S. malacoxylon* and the other plant species cause an enhanced absorption of calcium and phosphorus¹³⁻¹⁶ and this could account for the frequently observed hypercalcemia and hyperphosphatemia in affected animals, as well as the disease itself. The calcitonin-producing cells of the thyroid (i.e., the "C" cells) also show evidence of hyperactivity, an expected result of the hypercalcemic state.¹⁷ Analogy between the effects of the toxic plant and hypervitaminosis D has frequently been made. However, it was shown that the unknown factor seems to act faster and fade faster than a massive dose of vitamin D¹⁵ but appeared to cause an increase in calcium-binding activity in rabbit ileum.¹⁸

An understanding of the physiological basis of "Enteque seco" derives directly from current knowledge of vitamin D metabolism. It is now established that cholecalciferol (vitamin D₃), once acquired by the body, is converted into more polar metabolites that are more biologically active than vitamin D per se.^{19,20} The first important reaction occurs in the liver in which the vitamin is converted to the 25-hydroxylated derivative [25-(OH)D₃] and subsequently hydroxylated in the 1 α -position in the kidney to produce 1 α ,25-(OH)₂D₃. The 1 α ,25-(OH)₂D₃ metabolite is the most active of the cholecalciferol derivatives yet identified and, importantly, its rate of formation appears to be directly related to the calcium needs of the animal. As an example, when a calcium-deficient diet is fed, the recipient animal in some fashion can detect this

circumstance and increase the amount of 1 α ,25-(OH)₂D₃ produced by the kidney enzymes. As a consequence, the efficiency of calcium absorption is increased. On the other hand, an intake of a diet containing adequate levels of calcium and phosphorus reduces the amount of 1 α ,25-(OH)₂D₃ formed and this is followed by a decrease in the intestinal transport mechanism. Thus, there is a feed-back regulation of the activity of the 1 α -hydroxylase system of the kidney, which, through the controlled production of 1 α ,25-(OH)₂D₃, attempts to assure that either a sufficient amount of calcium is absorbed to meet physiological needs or that there is not an over-abundant absorption of this element.* If 1 α ,25-(OH)₂D₃ (or a compound that acts like this active metabolite) is administered, the control mechanism is circumvented and calcium absorption would proceed at a rate related to the amount of administered 1 α ,25-(OH)₂D₃ rather than to the calcium needs of the animal.

The etiology and symptoms of "Enteque seco" and related diseases suggested that the active principle in the calcinotic plants has biological properties similar to that of the active metabolite of vitamin D, i.e., 1 α ,25-(OH)₂D₃. This hypothesis was tested in chicks fed a high stable-strontium diet, a situation shown to inhibit the conversion of 25-(OH)D₃ to 1 α ,25-(OH)₂D₃ by the kidney enzymes.¹⁹ As a consequence, the absorption of calcium is depressed and the synthesis of the vitamin D-induced calcium-binding protein is inhibited²¹ and, as expected, strontium-inhibited chicks respond to 1 α ,25-(OH)₂D₃ but not to vitamin D₃ or 25-(OH)D₃.¹⁹ When fed

*This is a simplistic view of the intestinal phase of calcium homeostasis which hopefully suffices for the discussion of the calcinogenic plants. It should be recognized that the mechanism of control of the kidney 25-hydroxycholecalciferol-1 α -hydroxylase system is complex and certainly is not completely resolved at this time, particularly with regard to the primary endogenous and exogenous modulating factor or factors. For a fuller discussion of the problem, refer to references 19 and 20. The summary article in *Nutrition Reviews* 32:257-266, 1974, by M. R. Haussler on vitamin D metabolism is also highly recommended for this purpose.

either ground leaf power of *S. malacoxylon* or an extract thereof, the inhibitory effect of strontium was overcome both with regard to the enhancement of the intestinal absorption of calcium and the stimulation of the synthesis of the vitamin D-induced calcium-binding protein. This indicated that these plants do contain a substance that can mimic the action of $1\alpha,25\text{-(OH)}_2\text{D}_3$.^{22,23} A subsequent study by Walling and Kimberg (private communication) confirmed the $1\alpha,25\text{-(OH)}_2\text{D}_3$ -like property of *S. malacoxylon* since an extract of that plant proved effective in stimulating intestinal calcium transport in nephrectomized rats.

The evidence that the *S. malacoxylon* factor mimics the biological activity of $1\alpha,25\text{-(OH)}_2\text{D}_3$ allows an explanation of the etiology of the disease. As mentioned previously, a key reaction in calcium homeostasis is the rate of conversion of 25-(OH)D_3 to $1\alpha,25\text{-(OH)}_2\text{D}_3$ by the kidney enzyme system. Since the *S. malacoxylon* factor has biological properties similar to $1\alpha,25\text{-(OH)}_2\text{D}_3$, the point of regulation is essentially by-passed and calcium and phosphate absorption proceeds most efficiently and essentially out of control. The excessively absorbed calcium and phosphorus presumably cannot be physiologically accommodated, causing hypercalcemia, hyperphosphatemia, hypersecretion of calcitonin, and calcinosis. A direct corollary of this hypothesis is that the production of calcinosis in these animals requires two conditions: (a) the ingestion of the toxic plant and (b) an adequate dietary intake of calcium and phosphorus. Without both conditions, the disease is unlikely to occur.

Additional investigations on *S. malacoxylon* in chicks indicated that the vitamin D_3 -equivalents contained in the dried leaf is about 300,000 IU per kilogram.²³ This is a considerably greater concentration than reported for any other plant. Whether the formation of the vitamin D-like substance in *S. malacoxylon* requires ultraviolet irradiation is not exactly known. It was also shown that, in the rachitic chicks, the

enhancement of calcium absorption and induction of CaBP synthesis follow a time pattern of response reminiscent of that seen with $1\alpha,25\text{-(OH)}_2\text{D}_3$.²⁴ Although there was a somewhat greater lag period.²³ The maximum effect on calcium absorption occurred at 36 hours but returned to baseline at 48 hours.²³ CaBP levels also maximized at 36 hours; however, CaBP was still detectable at 48 hours and even at 72 hours. Again this is similar to the rachitic chick's response to $1,25\text{-(OH)}_2\text{D}_3$.²⁴

Whether the *S. malacoxylon* factor, like $1\alpha,25\text{-(OH)}_2\text{D}_3$, acts on the skeleton as well as the intestine, is controversial at this point. C. A. Mautalen¹⁵ injected ^{45}Ca into rabbits and observed that, at 30 days post-injection, the urinary excretion of ^{45}Ca was increased by a factor of about 5 after an extract of *S. malacoxylon* was administered. This indicated that the bone mobilization process was accelerated by *S. malacoxylon*. C.M. Campos et al.²⁵ fed a diet "practically free of calcium" to vitamin D-deficient rats and seven days later injected an extract of *S. malacoxylon* subcutaneously. Serum calcium concentrations became elevated at 24 hours and maximized at 48 hours, confirming the osteolytic effect of the active principle. There was also an early transient increase in serum phosphate levels at 6 hours. Campos et al.²⁵ further noted that the *S. malacoxylon* factor was effective in parathyroidectomized animals. However, contrary to the findings of Mautalen and colleagues,^{15,25} A. Uribe et al.²⁶ were unable to demonstrate an increase in serum calcium levels when the extract was administered orally to calcium-deficient and vitamin D-deficient rats and therefore it was concluded that, unlike vitamin D, the *S. malacoxylon* factor does not stimulate bone resorption. The only obvious difference between the Campos and Uribe studies was the route of administration of the extract and this might have a bearing on site of action. Additional investigations on this aspect are certainly required.

The question of whether a modification of the *S. malacoxylon* factor is a prerequisite for biological activity is of considerable interest. Since the factor is effective in chicks in which the kidney 1α -hydroxylase enzyme is suppressed by a high strontium intake or in nephrectomized rats, hydroxylation in the 1α -position is not required. Further, the *S. malacoxylon* factor is capable of stimulating calcium transport and inducing the synthesis of the vitamin D-dependent calcium-binding protein in an intestinal organ culture system.²⁷ Therefore, if any modification of the factor is indeed necessary, the intestinal tissue must provide the necessary enzymes to carry out the required reaction.

It has been claimed that preincubation of the *S. malacoxylon* extract with rumen liquor is necessary for biological effectiveness in rats²⁸ but others, as mentioned previously, were able to observe effects on calcium absorption in the rat without such pre-treatment.

As to the nature of the calcinotic principle in *S. malacoxylon*, the isolation and characterization of the active factor or factors has not been reported but a few properties are known. The factor in *S. malacoxylon* is a very polar molecule, being soluble in water, but insoluble in absolute ethanol, methanol:chloroform (2:1), chloroform, etc.^{1,23,29} It appears to be electrically neutral and its molecular size might be in the 1000-3000 range, i.e., larger than the cholecalciferol molecule.^{23,29} Because of its solubility properties, it was proposed that the *S. malacoxylon* factor might be a glycoside or contain some other group that accounts for its solubility. At any rate, the active principle is not $1\alpha,25\text{-(OH)}_2\text{D}_3$ per se as surmised from differences in solubility properties and apparent molecular size. However, since the factor does have the capacity to induce the synthesis of CaBP, the effective part of the molecule most likely has the appropriate steric configuration to interact with the protein synthetic machinery of the intestinal cell. The action of $1\alpha,25\text{-(OH)}_2\text{D}_3$ on calcium absorption by chick intestine in

vivo³⁰ and in organ culture is inhibitable by actinomycin D, an inhibitor of genetic transcription.²⁷ In the embryonic intestinal organ culture system, actinomycin D also inhibits the effect of *S. malacoxylon* on ^{45}Ca uptake and CaBP synthesis. The initial steps in vitamin D-induced protein synthesis involve the complexation of $1\alpha,25\text{-(OH)}_2\text{D}_3$ to cytoplasmic and nuclear receptors which accounts, at least in part, for the specificity of the synthetic process.³¹

All-in-all, the above information suggests that, on the basis of solubility, the *S. malacoxylon* factor is chemically different from $1\alpha,25\text{-(OH)}_2\text{D}_3$ but the biologically active fragment of the botanical substance might have some features in common with $1\alpha,25\text{-(OH)}_2\text{D}_3$. The eventual isolation and characterization of the active substances in these plants will indicate whether or not they do contain a chemical structure analogous to $1\alpha,25\text{-(OH)}_2\text{D}_3$. If these compounds do not, this could prove of considerable importance to the understanding of the mechanism of action of vitamin D on calcium transport.

Finally, it should be mentioned that the calcinogenic substances in these toxic plants could have therapeutic value in human and animal disease states associated with the abnormal metabolism of calcium and the skeleton. In fact, when further studied, it is possible that these substances might turn out to be more advantageous than $1\alpha,25\text{-(OH)}_2\text{D}_3$ in certain clinical situations.

One further point is directed to plant physiologists: What function might these $1\alpha,25\text{-(OH)}_2\text{D}_3$ -like substances serve in these calcinogenic plants? Is there a connection between these active substances, mineral metabolism in the botanical world, and soil type? This should constitute a worthy project for the interested botanist. □

1. N. A. Worker and B. J. Carrillo, *Nature* (London) 215: 72-74, 1967
2. B. J. Carrillo and N. A. Worker, *Rev. Invest. Agropecu, INTA* 4: 9-30, 1967

3. J. Döbereiner, C. H. Tokarnia, J. B. D. De Costa, J. L. E. Campos, and M. D. Dayrell, *Pesq. agropec. bras., Ser. Vet.* 6: 91-117, 1971
4. G. Dirksen, P. Plank, A. Spies, T. Hänichen, and K. Dämmrich, *Deutsch. Tierärztl. Wschr.* 77: 321-337, 1970
5. T. Hänichen, P. Plank, and G. Dirksen, *Deutsch. Tierärztl. Wschr.* 77: 338-342, 1970
6. G. Dirksen, P. Plank, U. Simon, T. Hänichen, P. Daniel, and A. Spies, *Deutsch. Tierärztl. Wschr.* 81: 1-5, 1974
7. R. M. Arnold and I. H. Finchem, *J. Comp. Path.* 60: 51-64, 1950
8. R. M. Arnold, *Trop. Animal Health Prod.* 1: 75-84, 1969
9. F. T. Lynd, E. H. Willers, L. A. Weight, and P. W. Gebauer, *Am. J. Vet. Res.* 26: 1344-1349, 1965
10. E. Ross, C. F. Simpson, L. O. Rowland, Jr., and R. H. Harms, *Poultry Sci.* 50: 870-873, 1971
11. L. Krook, R. H. Wasserman, J. N. Shively, A. H. Tashjian, T. D. Brokken, and J. F. Morton, *Cornell Veterinarian* (in press)
12. R. H. Wasserman, L. Krook, et al. (in preparation)
13. H. R. Camberos and G. K. Davis, *Gaceta Vet. A.L.P.A. Mem.* 3: 31-39, 1969
14. H. R. Camberos, G. K. Davis, and M. I. Djafar, in *Trace Element Metabolism in Animals*, C. F. Mills, Editor, Pp. 369-373, E. & S. Livingstone, Edinburgh, 1970
15. C. A. Mautalen, *Endocrinology* 90: 563-567, 1972
16. B. F. Sansom, M. J. Vagg, and J. Döbereiner, *Res. Vet. Sci.* 12: 604-605, 1971
17. B. J. Carrillo, *Rev. Invest. Agropecu, INTA* 10: 65-77, 1973
18. A. W. Wase, *Fed. Proc.* 31: 708, 1972
19. J. L. Omdahl and H. F. DeLuca, *Physiol. Rev.* 53: 327-372, 1973
20. E. Kodicek, *Lancet* i: 325-329, 1974
21. R. A. Corradino, J. G. Ebel, P. H. Craig, A. N. Taylor, and R. H. Wasserman, *Calc. Tissue Res.* 7: 81-92, 1971
22. R. H. Wasserman, *Science* 183: 1092-1094, 1974
23. R. H. Wasserman, A. Bar, R. A. Corradino, A. N. Taylor, and M. Peterlik, *Proceedings 5th Parathyroid Conf., Oxford, England, July, 1974*, Excerpta Medica (in press)
24. R. H. Wasserman, A. N. Taylor, and C. S. Fullmer, *Biochem. Soc. Spec. Publ.* 3: 55-74, 1974
25. C. M. Campos, M. Ladizesky, and C. Mautalen, *Calc. Tiss. Res.* 13: 245-248, 1973
26. A. Uribe, M. F. Holick, N. A. Jorgensen, and H. F. DeLuca, *Biochem. Biophys. Res. Commun.* 58: 257-262, 1974
27. R. A. Corradino and R. H. Wasserman, *Nature* (in press)
28. O. P. Gaggino, P. D. Deshpande, and J. M. Tilley, *Rev. Invest. Agropecu, INTA* 4: 123-128, 1967
29. D. J. Humphreys, *Nature (New Biol.)* 246: 155-157, 1973
30. D. E. M. Lawson and J. S. Emtage, *Biochem. Soc. Spec. Publ.* 3: 75-90, 1974
31. P. F. Brumbaugh and M. R. Haussler, *Biochem. Biophys. Res. Commun.* 51: 74-80, 1973

GROWTH OF THE HUMAN BRAIN: SOME FURTHER INSIGHTS

A further analysis of human brains shows that active brain growth starts during the second trimester of pregnancy and extends well into the third or fourth postnatal year.

Key Words: malnutrition, brain development, DNA concentration, postnatal, fetal

The possible adverse effects of malnutrition on mental performance have gained increased attention in recent years. This has naturally led to an intensive study of brain development in fetal and early post-natal life, when the other ill-consequences of malnutrition are glaringly evident. Brain growth does not progress uniformly but shows definite periods of increased activity — the growth spurts — during which the brain is considered to be particularly vulnerable to nutritional insults.¹ M. Winick and A. Noble² suggested that undernutrition during this proliferative period may cause permanent stunting of brain growth. Considerable species variations have been reported in the timing of the brain growth; it being perinatal in primates and mostly postnatal in rodents.³ This is the factor underlying Dobbing's timely warning⁴ to exercise caution while extrapolating results from animal experiments to the human situation.

To more clearly delineate the temporal sequence of the various stages of human brain development, J. Dobbing and J. Sands⁵ recently studied human brains of various ages, both fetal and postnatal. One hundred and thirty-nine brains ranging from ten weeks gestational age to seven postnatal years and nine adult brains were analyzed. It was ensured that none of the abortions or deaths occurred from neuropathological lesions.

The active period of cell multiplication in the human brain starts very early during fetal life⁶ and extends well into the end of the first postnatal year.⁷ The present study shows that the assessment of cellularity, usually expressed as concentration of DNA can be erroneous, since the latter falls at a time when the cell number is actually rising. This point has to be taken into consideration when interpreting the adverse effects of any agent on brain growth. It appears that the total content of DNA in the brain alone can give a true picture of cell number. The cerebellum starts growing later than the other regions of the brain, but the growth reaches a plateau earlier. Thus the cerebellum has a more rapid rate of growth. Unlike the rest of the brain, DNA concentration increases with rising cell numbers in the cerebellum.

The brain growth spurt, a transient period of rapid brain growth, can be observed in all species. According to these authors it begins when the adult neuronal number has already been largely achieved. "Thus it begins at about birth in the rat and about midgestation in the human. The enormous multiplication of cells which occupies its early part is, predominantly, glial." The fact that cell multiplication occurs relatively early in pregnancy in the human should provide protection against adverse effects caused by maternal malnutrition whereas the rat would be more vulnerable since cell multiplication occurs much later. The phase of cell multiplication is followed by myelination and the estab-

lishment of synaptic connections. These processes are mostly postnatal, both in the rat and human, and in this respect the human resembles the rat more than the pig. The present study shows that in the human, this extends to the third postnatal year. It had not been recognized hitherto that the brain growth spurt extends over such a prolonged period in the human. This is a period when children in the developing countries are dangerously exposed to malnutrition. Thus, the effect of malnutrition on myelination and its significance may be important aspects to be investigated. In animal experiments the data obtained from animals whose temporal sequence of brain growth resembles that of man may be the most meaningful. □

1. J. Dobbing in *Malnutrition, Learning and Behavior*. N. S. Scrimshaw and J. E. Gordon,

Editors, p. 181-202, M.I.T. Press, Cambridge, Mass., 1968

2. M. Winick and A. Noble: Cellular Responses in Rats during Malnutrition at Various Ages. *J. Nutrition* 89: 300-306, 1966
3. A. N. Davison and J. Dobbing in *Applied Neurochemistry*. A. N. Davison and J. Dobbing, Editors, p. 253-286, Oxford Blackwell Scientific Publications, Oxford and Edinburgh, 1968
4. J. Dobbing: The Developing Brain: A Plea for More Critical Interspecies Extrapolation. *Nutrition Rep. Int.* 7: 401-406, 1973
5. J. Dobbing and J. Sands: Quantitative Growth and Development of Human Brain. *Arch. Dis. Child.* 48: 757-767, 1973
6. J. Dobbing and J. Sands: Timing of Neuroblast Multiplication in Developing Human Brain. *Nature* (London) 226: 639-640, 1970
7. M. Winick: Changes in Nucleic Acid and Protein Content of the Human Brain during Growth. *Pediat. Res.* 2: 352-355, 1968

INFANT BODY COMPOSITION BY SKINFOLD MEASUREMENTS

Careful use of Harpenden calipers on newborn infants allows measurements to be made of both subcutaneous fat and interstitial water.

Key Words: skinfold thickness, Harpenden calipers, subcutaneous fat, interstitial water, newborn infant, small-for-dates infant

Measurement of the body fat of infants is beset by technical and ethical difficulties that have hampered the collection of data which is pertinent to both prenatal and postnatal nutrition. For example, direct measurement by fat-soluble gas dilution techniques¹ or specific gravity measurements² are not applicable to the newborn. Other methods of measuring subcutaneous fat thickness such as radiology, ultrasound, and electrical conductivity do not distinguish between subcutaneous fat and interstitial water. Contrasting with these approaches is the use of special calipers to measure subcutaneous fat by the thickness of a double skinfold in certain selected sites

on the body.³ This method has the advantages of being rapid, noninvasive, and harmless, but suffers from large errors with the same observer and between observers.⁴ Part of the difficulty stems from the fact that the initial skinfold thickness decreases in the seconds after the calipers have been applied.

Y. W. Brans et al.⁵ turned this potential disadvantage to good effect by making carefully controlled measurements of skinfold thickness in different groups of newborn infants. This group has a longstanding interest in the distribution of body water in the newborn.⁶⁻⁸ They argued that the decrease in skin thickness following application of the calipers might result from the squeezing of water from the subcutaneous tissue.

Mid-triceps and subscapular skinfolds were measured within 24 hours of birth in 23 term infants of normal weight, 23 normally grown preterm infants, six small-for-dates term infants, and seven small-for-dates preterm infants. The babies were randomly selected from the hospital nursery and were otherwise healthy. Skinfold thickness was measured to the nearest 0.05 mm with Harpenden calipers³ at exactly 15 and 60 seconds after application by one of three observers. A preliminary study in which readings were taken every 10 seconds showed that the measurement became stable at 60 seconds. The change in thickness from 15 to 60 seconds was expressed as a percent of that at 15 seconds. The infants were weighed each day and the difference between their minimum weight and birthweight was expressed as a percent of the birthweight. The preterm infants of normal weight lost 13.4 percent of their birthweight whereas the other three groups lost between 4.4 and 5.8 percent. No explanation for this difference is given and the reader is left to conjecture if it might be due to differences in feeding practices.

The results from readings at the mid-triceps and subscapular sites were similar, so attention will be paid only to the mid-triceps results. In the infants of normal weight there was a good linear correlation between the skin thickness at 60 seconds and the birthweight or gestational age. When the small-for-dates babies were plotted in the same way, they followed the same regression line as the normally grown babies when birthweight was the variable, but fell below normal when gestational age was the variable. These results indicate that the small-for-dates babies had normal subcutaneous fat for their body weight which is somewhat surprising to the clinician who thinks of them as being characteristically long and thin.

The percentage fall in skin thickness between 15 and 60 seconds was inversely correlated with gestational age and in this correlation normal and small-for-dates infants behaved similarly. This function of skinfold thickness was independent of

birthweight above 2.5 kg but increased with diminishing birthweight below this figure. The same function correlated positively with the maximum postnatal percentage weight loss.

G. Cassady and his co-workers emphasize that skinfold thickness calipers are deceptively easy to use but have shown how, if used properly, they may give information not only on subcutaneous fat but also on interstitial water content. The possibility that the change in the measurement from 15 to 60 seconds is due to extravasation or compression of fat seems unlikely. The negative correlation between this measurement and the gestational age fits well with earlier observations of a fall in extracellular water with increasing maturity.

Earlier work had suggested that skinfold thickness of newborn infants was positively related to maturity⁹ and normality of intrauterine growth.¹⁰ Both points have been amply confirmed by the present study which should go a long way to reassure investigators that this approach does not lack precision when the measurement is performed correctly. Skinfold thickness measurements have been performed on post-natally malnourished infants.¹¹ It is obvious from the work reviewed here that the technique could be easily adapted so that the worker in the field might obtain information on both subcutaneous fat and water. When used on edematous patients, however, the posture of the subject must be taken into account in interpreting the readings. □

1. G.T. Lesser and G. Zak: Measurement of Total Body Fat in Man by the Simultaneous Absorption of Two Inert Gases. *Ann. N.Y. Acad. Sci.* 110:40-54, 1963
2. J. Parizkova: Total Body Fat and Skinfold Thickness in Children. *Metabolism* 10:794-807, 1961
3. J.M. Tanner and R.H. Whitehouse: The Harpenden Skinfold Caliper. *Am. J. Physiol. Anthropol.* 13:743-746, 1955
4. K.D.G. Edwards and H.M. Whyte: The Simple Measurement of Obesity. *Clin. Sci.* 22:347-352, 1962

5. Y.W. Brans, J.E. Sumners, H.S. Dweck, and G. Cassady: A Noninvasive Approach to Body Composition in the Neonate: Dynamic Skin-fold Measurements. *Pediat. Res.* 8:215-222, 1974
6. G. Cassady: Plasma Volume Studies in Low Birth Weight Infants. *Pediatrics* 38:1020-1027, 1966
7. G. Cassady: Bromide Space Studies in Infants of Low Birth Weight. *Pediat. Res.* 4:14-24, 1970
8. G. Cassady and R.R. Milstead: Antipyrine Space Studies and Cell Water Estimates in Infants of Low Birth Weight. *Pediat. Res.* 5:673-682, 1971
9. J. Gleiss and M. Hermanns: Ektodermale Kriterien zur Klinischen Reifesbestimmung neugeborenen. *Arch. Kindaheilk.* 179:266-283, 1969
10. R. Usher and F. McLean: Intrauterine Growth of Liveborn Caucasian Infants at Sea Level: Standards Obtained from Measurements in 7 Dimensions of Infants Born between 25 and 44 Weeks of Gestation. *J. Pediat.* 74:901-910, 1969
11. A.R. Frisancho and S.M. Garn: Skinfold Thickness and Muscle Size: Implications for Developmental Status and Nutritional Evaluation of Children from Honduras. *Am. J. Clin. Nutrition* 24:541-546, 1971

FOLATE BINDER IN LEUKOCYTES AND SERUM

A macromolecular factor which binds unreduced and partially reduced folate but not tetrahydrofolate has been detected in the leukocytes and serum of patients suffering from chronic myelogenous leukemia, pregnant women, and women using oral contraceptives. Its physiological (or pathological) role remains speculative.

Key Words: cell lysates, folic acid, folate binder, macromolecular

A macromolecular factor which binds with unreduced folate and dihydrofolate (FH_2) but not tetrahydrofolate (FH_4) was accidentally discovered by S. P. Rothenberg and co-workers^{1,2} in some leukemic cells while assaying the folate reductase activity of those cells using a newly developed radioassay. Trace amounts of ^3H -folic acid are used as the substrate in this assay and the radio-activity in FH_4 after chromatographic separation of the product is measured. Thus, it obviates the use of high concentrations of substrate, which are required for the conventional spectro-photometric assay.

Little or no folate reductase activity could be detected in the cell lysates from four out of seven patients suffering from chronic myelogenous leukemia (CML) when tracer concentration ($1.14 \times 10^{-9}\text{M}$) of the substrate ^3H -folic acid was used.

However, when the substrate concentration was raised by the addition of $0.22 \times 10^{-6}\text{M}$ stable folic acid, reduction of ^3H -folic acid became apparent. With a further increase in the concentration of stable folate, an isotope dilution effect was evident. In the case of cell lysates having normal enzyme activity, the curve for isotope dilution could be extrapolated to zero concentration of cold folate.

The peculiar behavior of lysates from CML patients was also apparent when they were mixed with lysates of cells having normal enzyme activity. This suggested the presence of a material in CML cells, which bound the trace amount of labeled folate and made it unavailable for enzymatic reduction. Addition of stable folate displaced the ^3H -folate from the binder and reduction was evident.

Gel filtration of the cell lysates from CML patients preincubated with ^3H -folate indicated that the binder was a macromolecule having a molecular weight of

100,000 to 200,000. It was found to be resistant to heating at 56°C for 30 minutes and was minimally active below a pH of 5.0. It bound folic acid and FH_2 rapidly and reacted with such reduced folate analogues as diapterin, pteropterin, and methotrexate. Its presence could also be detected in the serum, probably due to liberation from the cells that were destroyed.

The folate binder could not be detected in all leukemic diseases, but that does not rule out its presence since endogenous folate may bind it and render it undetectable by the "isotope binding" method.

In a more recent paper, M. Da Costa and S. P. Rothenberg³ demonstrated the presence of this factor in the leukocytes of pregnant women as well as of women taking oral contraceptive agents (OCA). It seems to be induced by the hormonal changes associated with pregnancy and use of OCA. Sixty-four women in the third trimester of pregnancy, ten women taking OCA, and 15 non-pregnant menopausal women were examined. Lysates or sera binding less than 5 percent ^3H -folic acid (PGA) were considered to contain no folate binder.

Of the 64 pregnant women studied, 31 had lysates which bound more than 5 percent of the labeled folate, with a mean \pm SEM binding of 38 ± 5 percent. Seven out of ten women on OCA had a detectable binder with a mean of 22 ± 8 percent. None of the lysates from non-pregnant women bound ^3H -PGA. There was a marked fall in the binding of ^3H -PGA by cell lysates after parturition.

The hormone induced folate binder was also a macromolecule and could react with the anti-sera for CML binder. The anti-sera were obtained by immunizing rabbits with cell lysates of CML cells containing a high concentration of the binder. This shows that the folate binder from leukemic cells is immunologically similar to the hormonally induced binder and that it is not just peculiar to malignant cells.

Levels of several proteins are known to rise in pregnancy and in women using OCA.

It is possible that this factor is a normal constituent of cells and serum, but remains saturated by the endogenous folate. In folate deficiency or in conditions where the level of the binder increases, its presence can be detected by the ^3H -folate binding test. The presence of folate binder(s) in the sera of folate deficient subjects has been reported.³

The physiological or pathological implications of this macromolecular factor are not clear. Since it binds FH_2 , it may have some function in de novo thymine-DNA synthesis. Da Costa et al.⁴ observed that CML cells containing a high concentration of the folate binder possess reduced capacity for the methylation of deoxyuridylate to thymidylate. Since the factor can also bind with methotrexate (a potent inhibitor of folate reductase), it may affect the treatment of leukemia by methotrexate and may be the basis of drug resistance shown by some leukemic cells. In such cases an appropriate adjunct to therapy might be simultaneous administration of folic acid which by virtue of its greater affinity for the binder would leave methotrexate to bind with the enzyme folate reductase. The folate binding factor may have potential value in the treatment of neoplastic diseases, provided it could enter the cell.

Biochemical evidence of folic acid deficiency, as judged by low serum folate levels and elevated FIGLU excretion, is quite common in pregnancy and among women using OCA. Cases of megaloblastic anemia have been reported among these women, particularly during pregnancy. Although Da Costa and Rothenberg³ could not observe a direct correlation between the concentration of folate binder and serum folate or hemoglobin, nor did any of the women studied have megaloblastic anemia, the percent of pregnant women showing serum folate concentration above 5 ng per milliliter was higher in the absence of the binder (63 percent) than in the presence of the binder (38 percent). Based on this, the authors speculate that "increased synthesis of this binder in prolifera-

ting cells such as bone marrow could sequester FH_2 from a metabolically active pool of folate coenzymes. Under such circumstances this could potentiate the metabolic consequences of inadequate folate intake or increase folate demands." □

1. S. P. Rothenberg: A Macromolecular Factor in Some Leukemic Cells Which Binds Folic Acid. *Proc. Soc. Exp. Biol. Med.* 133: 428-432, 1970

2. S. P. Rothenberg and M. Da Costa: Further Observations on the Folate-Binding Factor in some Leukemic Cells. *J. Clin. Invest.* 50: 719-726, 1971
3. M. Da Costa and S. P. Rothenberg: Appearance of Folate Binder in Leukocytes and Serum of Women Who are Pregnant or Taking Oral Contraceptives. *J. Lab. Clin. Med.* 83: 207-214, 1974.
4. M. Da Costa, S. P. Rothenberg, and B. Kamen: DNA Synthesis in Chronic Myelogenous Leukemic Cells: Comparison of Results in Cells Containing Folate Binding Factor to Replicating Cells without Binder. *Blood* 39: 621-627, 1972

THE CORRELATION OF SERUM FERRITIN AND BODY IRON STORES

The relationship between serum ferritin levels and total body iron stores is discussed. Recent work is evaluated which correlates these parameters in iron deficiency, liver disease, inflammation, renal failure, and states with increased red cell turnover.

Key Words: serum ferritin, body iron stores, hepatocellular disease, chronic inflammation

The assessment of total body iron stores has long lacked an easy method of measurement. The differential desferrioxamine test,¹ where iron chelated and excreted is thought to be proportional to body iron stores, requires a fair degree of cooperation from the subject studied and there is evidence that some of the iron chelated is not derived from iron stores.² The most commonly used method is assessment of the amount of iron present in the bone marrow but this is an uncomfortable procedure and at best only semi-quantitative. Quantitative venesection to the level of incipient iron deficiency and anemia is sensitive³ but impracticable. Certainly none of these methods is suitable for large scale population surveys.

In 1972, G. M. Addison and his colleagues published a sensitive radioimmunoassay for serum ferritin.⁴ Recently a 'two-

site' immunoassay has also been evolved.⁵ In this modification, unlabeled antibody linked to an immunoadsorbent couples with ferritin which then reacts with labeled antibody. Thus quantitation of label bound is a measure of ferritin concentration. It has become apparent that in many instances serum ferritin concentration is related to the total body iron stores.^{4,6}

Ferritin is a high molecular weight compound composed of a protein shell of apoferritin synthesized by the liver surrounding a ferric hydroxide core, the whole forming an octahedral structure. It is the main storage form of iron in the liver and reticulo-endothelial system either as ferritin itself, or as hemosiderin which is thought to be a complex aggregate of partially denatured ferritin. In contrast to the iron stores (1 g in a normal adult male) the amount of ferritin found in the serum by radioimmunoassay is miniscule. The normal range for the two site assay is generally

accepted as 12 to 300 ng per milliliter.⁵ In normal adults the known difference in iron stores between the sexes is reflected in the serum levels of ferritin. In one series of non-anemic individuals the geometric mean of serum ferritin levels was 140 ng per milliliter for males and 39 ng per milliliter for females.⁴ Previous surveys found levels below 10 ng per milliliter in iron deficiency⁴ and supra-normal levels in states of iron overload.⁷ Serum ferritin levels fall with repeated venesection⁸ and in refractory anemias are proportional to the number of transfusions received.⁶

The main value of the immunoassay method is its ease, and its requirement of only small amounts of serum or plasma which make it eminently suitable for large scale surveys, e.g. of iron nutritional status in a population or the effect thereon of iron additives to food. However, it has also been claimed to be of value in discriminating between true iron deficiency and the anemia complicating chronic infection or chronic inflammatory disorders. In both these situations the serum iron levels will be low and there is likely to be a microcytic hypochromic anemia. Some distinction between the two conditions may be found in the total iron-binding capacity which is usually raised in iron deficiency and low in the anemia of chronic disorders although there are exceptions. Assessment of bone marrow stores will usually provide the answer. There are, of course, therapeutic implications in assessing iron stores since the anemia of chronic disorder,⁹ even if hypochromic and microcytic, will respond little to iron therapy.

Since apoferritin is synthesized in the liver and ferritin is stored in large amounts therein, it seems probable that hepatic function could also affect the serum ferritin levels. A series of hospital patients has now been studied by D.A. Lipschitz and his co-workers¹⁰ and the levels of serum ferritin correlated with other hematological parameters. Serum iron, total iron-binding capacity, and serum ferritin were measured in all subjects. Thirty-two patients with uncomplicated iron de-

ficiency were studied. The criteria for selection were a transferrin saturation of less than 16 percent (8 ± 3 percent for the group), a total iron-binding capacity of greater than 400 ng per 100 ml (actually estimated as 479 ± 73 ng per 100 ml), and no evidence of an inflammatory or hepatic disorder. In this group the mean serum ferritin was found to be 4 ng per milliliter with a range of 1 to 14 ng per milliliter. Conversely, in iron overloaded patients, high serum ferritin levels were found. In three patients with hemochromatosis untreated by venesection the values were 3215, 6018, and 6100 ng per milliliter. In 20 patients who had received at least 20 blood transfusions the mean serum ferritin was 2713 ng per milliliter. A highly significant correlation was observed between the number of transfusions and the serum ferritin level, and analysis of the data showed that each transfusion raised the serum ferritin by the order of 60 ng per milliliter.

Thirty-nine patients were classified as having an inflammatory disorder on the grounds of clinical symptoms, erythrocyte sedimentation rate, pyrexia, or leucocytosis. Some of these patients were anemic with a mean hematocrit of 30 ± 4 percent. Low serum iron levels were found, 38 ± 11 μ g per 100 ml, but total iron-binding capacity was low at a mean of 227 ± 81 μ g per 100 ml. Transferrin saturation, therefore, was higher than that in iron deficiency at 18 ± 5 percent. In this group the mean serum ferritin was 305 ng per milliliter with a wide range of 10 to 1650 ng per milliliter. There was no apparent relation between serum ferritin and the duration, type, or severity of the inflammatory process.

Thirty-seven patients had liver disease as assessed by increased serum bilirubin and high serum alkaline phosphatase levels. The majority had an alcoholic etiology and all these patients were anemic. The hematocrit of the group as a whole was 30 ± 4 percent. This group had high serum iron levels, slightly reduced total iron-binding capacity, and high transferrin saturation. These patients had a high serum ferritin level with a mean of 509 ng per milliliter and a range

of 25 to 3239 ng per milliliter. The mean values of the 29 patients with alcoholic liver disease were comparable with those of eight patients with viral hepatitis of whom only one was anemic. Nine additional patients had both liver dysfunction and evidence of an inflammatory process. Here the serum ferritin was higher still with a mean of 801 ng per milliliter and again with a wide range.

Seventy-five of the patients in the inflammatory and hepatic dysfunction groups and a control group consisting of patients with other disorders had bone-marrow aspirations performed for the assessment of iron stores. These were categorized as absent, diminished, moderate, or increased and correlated with the basic diagnosis. Patients with inflammation showed elevation of the serum ferritin compared with the control group for a given amount of marrow iron. This was even more true of patients with liver disease. Thus, of those with absent iron stores, the 12 control patients had mean serum ferritin levels of 6 ng per milliliter, the patients with inflammation 21 ng per milliliter, and the patients with liver disease 61 ng per milliliter. A similar pattern of results was seen at each level of bone marrow iron stores. Thus a low serum ferritin level provides evidence of iron deficiency, but the converse that a normal level excludes it, is not shown to be so. Additionally an increased serum iron level may be due to inflammation or hepatic disease, rather than to increased iron stores. In these two groups the few patients with additional iron deficiency did tend to have lower serum ferritin levels than those without iron deficiency but the majority still fell within the normal range.

Fifteen patients studied had an anemia with increased red cell turnover; seven patients with megaloblastic anemia, one with sideroblastic anemia, and eight with a hemolytic anemia. In this group the mean serum ferritin was high at 419 ng per milliliter. Twelve of these patients had bone marrow aspirations. As with the previous groups, there was a tendency for serum ferritin levels to be higher than normal for

a given quantity of bone marrow iron. For example, four patients with diminished iron stores had serum ferritin values of 61 to 354 ng per milliliter. In nine patients with chronic renal disease who had not received iron therapy or blood transfusion, the mean serum ferritin was 32 ng per milliliter and levels showed a correlation with bone marrow iron stores comparable to the control group.

A correlation was observed in these studies between serum ferritin and total iron-binding capacity but not with any other hematological parameter. This was particularly true for 39 patients with anemia and inflammation. The correlation is, of course, inverse with iron overloaded patients showing the highest serum ferritin and lowest total iron-binding capacity and iron deficiency showing converse results. This is an interesting observation and the authors suggest that total iron-binding capacity should receive more attention as a possible measure of total body iron stores.

This paper thus substantiates the hypothesis that serum ferritin will delineate uncomplicated iron deficiency. Iron deficiency compounded by hepatic disease or chronic inflammation will not necessarily be manifest in the serum ferritin levels. Since a similar high ferritin was also found in hemolytic states, this presumably will also be the case in the uncommon situations where iron deficiency and hemolysis can co-exist, e.g. paroxysmal nocturnal hemoglobinuria. The conclusion suggested by this work is that in a patient with chronic inflammation a high serum ferritin will exclude iron deficiency but a result within the normal range will not. This aspect requires further investigation in a larger number of patients. It would also be of interest to investigate other anemias thought to be due to a block to iron release by the reticulo-endothelial cell, e.g. rheumatoid arthritis and neoplasias, particularly of the gut where there may be an associated iron deficiency due to blood loss.

The alcoholic patients with hepatic dysfunction included in this study were all

anemic and it would be of interest to have been given details of the etiology of the anemia. In this situation it is often a megaloblastic anemia due to a dietary deficiency of folate or a secondary sideroblastic anemia. These are both conditions of increased red cell destruction and it might have been thought that some of the increase in serum ferritin levels could be attributable to this. However, although their number is small, the hepatitis patients of whom only one was anemic, showed comparable elevation of serum ferritin levels.

Recent work¹¹ where the ferritin and transferrin fractions of serum were separated by ultracentrifugation after the administration of ⁵⁹Fe-labeled heat-damaged red cells suggested that serum ferritin iron is largely derived from reticulo-endothelial iron. Normally this iron comes from senescent red cells. Ferritin iron is then largely transported to the hepatocytes. Transferrin, on the other hand, is concerned with transfer of iron from the liver and after absorption from the gut to the developing erythropoietic cells. If these results are substantiated it seems notable at first sight that in situations where the reticulo-endothelial system fails to release iron, nevertheless, supra-normal levels of ferritin, whose iron moiety is thought to be derived from this same source, are detectable in the plasma. However, more rapid turnover of apoferritin from damaged liver cells is likely in hepatocellular disease, but this is also a possibility in chronic inflammatory states where there are increases in other proteins synthesized in the liver, e.g. fibrinogen and haptoglobin. The development of methods for measuring ferritin have obviously opened up a new chapter in the field of iron metabolism and the next few years should see substantial advances. □

1. J. Fielding, M. C. O'Shaughnessy, and G. M. Brunström: Differential Ferrioxamine Test in Idiopathic Haemochromatosis and Transfusional Haemosiderosis. *J. Clin. Path.* 19: 159-164, 1966
2. K. S. Olsson: Iron Stores in Normal Men and Male Blood Donors. As Measured by Desferrioxamine and Quantitative Phlebotomy. *Acta Med. Scandinav.* 192: 401-407, 1972
3. D. Haskins, A. R. Stevens, Jr., S. Finch, and C. A. Finch: Iron Metabolism. Iron Stores in Man As Measured by Phlebotomy. *J. Clin. Invest.* 31: 543-547, 1952
4. G. M. Addison, M. R. Beamish, C. N. Hales, M. Hodgkins, A. Jacobs, and P. Llewellyn: An Immunoradiometric Assay for Ferritin in the Serum of Normal Subjects and Patients with Iron Deficiency and Iron Overload. *J. Clin. Path.* 25: 326-329, 1972
5. L. E. M. Miles, D. A. Lipschitz, and C. P. Bieber: *Anal. Biochem.* in press
6. A. Jacobs, F. Miller, M. Worwood, M. R. Beamish, and C. A. Wardrop: Ferritin in the Serum of Normal Subjects and Patients with Iron Deficiency and Iron Overload. *Brit. Med. J.* 4: 206-211, 1972
7. M. A. Siimes, J. E. Addiego, and P. R. Dallman: Ferritin in Serum: Diagnosis of Iron Deficiency and Iron Overload in Infants and Children. *Blood* 43: 581-590, 1974
8. G. O. Walters, F. M. Miller, and M. Worwood: Serum Ferritin Concentration and Iron Stores in Normal Subjects. *J. Clin. Path.* 26: 770-772, 1973
9. G. E. Cartwright and G. R. Lee: The Anaemia of Chronic Disorders. *Brit. J. Haematology* 21: 147-152, 1971
10. D. A. Lipschitz, J. D. Cook, and C. A. Finch: A Clinical Evaluation of Serum Ferritin as an Index of Iron Stores. *New Engl. J. Med.* 290: 1213-1216, 1974
11. M. A. Siimes and P. R. Dallman: New Role for Serum Ferritin (SF) in Iron Metabolism. *Pediat. Res.* 8: 409, 1974

NUTRITION CLASSICS

from THE JOURNAL OF PHYSIOLOGY 35:88-102, 1906-1907

THE IMPORTANCE OF INDIVIDUAL AMINO-ACIDS IN METABOLISM. Observations on the Effect of adding Tryptophane to a Dietary in which Zein is the sole Nitrogenous Constituent. By EDITH G. WILLCOCK, *Fellow of Newnham College, Cambridge*, and F. GOWLAND HOPKINS, F.R.S.

(From the Physiological Laboratory, Cambridge.)

THE behaviour of the animal body under the influence of deficiencies in its supply of nitrogenous foodstuffs has hitherto been studied almost entirely from the aspect of fluctuations in nitrogenous equilibrium. It is, of course, clear that the existence of such equilibrium, if sufficiently prolonged, is the best proof that all the needs of the body are being satisfied. It is no less certain, however, that the full significance of nitrogenous equilibrium is as yet but imperfectly understood. Many metabolic factors are probably involved in its maintenance, and the condition is one which should be susceptible of some experimental analysis.

A deficiency in a nitrogenous dietary need not necessarily be one of quantity; the form in which the nitrogen is supplied may determine its efficiency. Thus, in the familiar case of gelatine it is, of course, a qualitative deficiency which makes that substance unable to maintain nitrogenous equilibrium. It is generally supposed that this qualitative deficiency is occasioned by the absence from gelatine of certain molecular groups which are present in true protein, but this hypothesis leaves unexplained the advantage possessed by gelatine over fats and carbohydrates as a protein sparer. It is assumed that gelatine, owing to its constitutional deficiencies, cannot repair tissue waste, but can replace protein in so far as the latter functions as a source of energy; sharing with the proteid some unexplained advantage over fats and carbohydrates in this latter capacity.

Recent advances in physiology seem to justify a fresh attack upon this subject, upon somewhat different lines. It now seems necessary to

differentiate between the minimal amount of protein necessary for actual tissue repair and that required for total maintenance; we have no reason for assuming that they are the same. We are no longer bound to Liebig's view, or to any modification of it which implies that the whole of the proteid consumed is utilized as intact proteid; nor are we even compelled to assume that the whole of what is broken down in the gut is resynthesised before utilization. Proteid products may function in other ways than in the repair of tissues or in supplying energy. It is highly probable that the organism uses them, in part, for more specific and more immediate needs. The discovery of substances absolutely essential to life, highly specific, and elaborated in special organs, suggests that some part, at least, of the protein products set free in the gut may be used directly by these organs as precursors of such specific substances. In adrenaline, for instance, we have an aromatic substance absolutely essential for the maintenance of life, and it is probable that the suprarenal gland requires a constant supply of some one of the aromatic groups of the proteid molecule to serve as an indispensable basis for the elaboration of adrenaline. If this be so it is certain that the gland itself could not, in starving animals, supply sufficient of such a precursor to outlast the observed survival periods¹.

• • •

On this basis we have a hypothesis to account for the special protein-sparing properties of gelatine. It shares with protein certain molecular groupings needed to satisfy specific needs, and is thus superior to fats and carbohydrates as a protein-sparer; it lacks, on the other hand, certain other necessary groupings, fails therefore to supply all such needs, and thus cannot replace true proteid.

Considerations such as these formed the basis for the experiments described in the present paper. The results obtained serve to show that even when tissue-equilibrium is not maintained the presence or absence of some one amino-acid in the diet may affect most materially the survival period and general well-being of an animal.

Exp. IV; Series E, F, and G. In this experiment three series were compared. The one had the zein diet alone; another had the same with tryptophane added to the extent of 2 per cent of the zein present. The third series had the zein diet with tyrosine added to the extent of 2% of

the zein. Tyrosine is already present abundantly in zein, and on this account it was chosen as an addendum to a series forming a second control. That in this, as in subsequent experiments, its addition makes no difference to the effect of the zein diet, is sufficient to show that the modifications due to tryptophane are specific and not due merely to increasing the amount of aromatic amino-acids.

E. Zein diet.			F. Zein diet+Tyrosine.			G. Zein diet+Tryptophane.		
Mouse No.	Survival period	Change in weight	Mouse No.	Survival period.	Change in weight	Mouse No.	Survival period	Change in weight
19	15 days	-27.7 %	26	12 days	-24.3 %	33	24 days +	40.4 % (alive)
20	15	-32.2	27	15	-31.5	34	24 +	39.0 (alive)
21	15	-20.3	28	19	-30.3	35	24 +	38.0 (alive)
22	17	-32.6	29	19	-27.7	36	24 +	27.6 (alive)
23	12	-22.7	30	10	-27.6	37	24 +	28.8 (alive)
24	17	-36.5	31	10	-17.3	38	16	33.7
25	17	-32.9	32	12	-32.8	39	10	34.8
Mean	15	-29.2		14	-27.3		21 + +	34.6

The experiment had, unfortunately, to be stopped on the 24th day. By this time all the mice without tryptophane had been dead for some days, while of the seven which received tryptophane five were still alive, and, though thin, were well and active. The temperature when the experiment was made was low, and all the mice without tryptophane were extremely torpid after exposure to cold in the night. They had to be warmed in front of a fire in the morning before becoming sufficiently active to seek their food. The tryptophane series were warmed at the same time to keep the conditions of experiment similar, but in their case there was no necessity for this; they were active throughout.

• • •

SUMMARY OF RESULTS.

1. A dietary containing zein as its only nitrogenous constituent is unable to maintain growth in young mice.
2. The addition of tryptophane (an amino-acid absent from the decomposition products of zein) to such a dietary does not make it capable of maintaining growth.
3. On the other hand this addition greatly prolongs the survival period of animals fed upon zein, and materially adds to the well-being of such animals.
4. The addition of tyrosine (which is already present in zein), in equivalent amounts, has no such effect.
5. It is suggested that the tryptophane is directly utilized as the normal precursor of some specific "hormone" or other substance essential to the processes of the body.

ZINC AVAILABILITY IN LEAVENED AND UNLEAVENED BREAD

*The leavening process was shown to greatly increase the solubility
and in vitro intestinal uptake of zinc from wholemeal bread.*

Key Words: zinc, phytate, wheat

A characteristic of the rural Iranian diet, which induces a high incidence of zinc deficiency, is the high proportion of unleavened wholemeal wheat bread which contains significant concentrations of phytate.

A role of phytate (inositol hexaphosphate) in interference with absorption of zinc in animals has been reported and apparently explained as the result of the formation of a highly insoluble zinc-calcium-phytate complex.¹ It is probable that in natural foodstuffs, substances other than phytate interfere with zinc absorption.² Since zinc supplementation of these diets in rural areas is probably not a practical solution to the problem, J. G. Reinhold et al.³ recently investigated the effects of leavening of bread on increasing the solubility of its zinc. This investigation follows earlier observations that the leavening process reduces the phytate content by actions of phytate-splitting enzymes of the yeast.

⁶⁵Zn-labeled wheat was produced in a greenhouse by injecting a ⁶⁵Zn chloride solution into the stems of plants ten days before maturity and harvest. The labeled wheat was ground and used after dilution with ordinary Iranian wholemeal flour for the production of leavened and unleavened bread. The latter contained killed yeast to balance the live yeast used in the leavening

process. The baked bread was dried and ground to a powder.

Solubility of ⁶⁵Zn and total zinc was measured in supernatant solutions following two-hour extractions of bread flours in 0.85 percent sodium chloride adjusted to a pH of 4.5 to 7.5.

⁶⁵Zn uptake was measured in opened segments of rat small intestine incubated aerobically in a sodium chloride-potassium chloride-glucose solution adjusted to a pH of 6.0 to 6.6 and containing the bread flours. After incubation the intestinal segments were gently rinsed, dissolved in sulfuric acid, and subjected to gamma counting.

Phytate was determined as the phosphate present in precipitated ferric phytate after its complete hydrolysis.

Solubility tests measured either as ⁶⁵Zn or total zinc released gave similar results. As the pH was lowered from 7.5 to 4.5, solubility of ⁶⁵Zn increased exponentially. At all pH levels the ⁶⁵Zn from leavened bread flour was more soluble than that from unleavened bread flour. The difference was about threefold between a pH of 6.0 and 7.0, the range common in the small intestinal lumen.

Uptake of ⁶⁵Zn by five of the six segments of jejunum and ileum tested was significantly increased by 30 to 50 percent when leavened bread flour was tested in comparison to the unleavened bread flour.

The leavening process decreased phytate content of the bread by 15 to 25 percent, increased ^{65}Zn solubility by three- to four-fold, and increased ^{65}Zn uptake by 30 to 50 percent. The disproportions among these data suggest that zinc availability may be mediated by factors other than phytate, either in the bread flour or inherent in the incubation system employed. In any event, it appears that leavening of wholemeal bread might result in improved utilization of the zinc in the bread. □

1. B. L. O'Dell: Effect of Dietary Components Upon Zinc Availability. *Am. J. Clin. Nutrition* 22: 1315-1322, 1969
2. J. G. Lease and W. P. Williams, Jr.: Availability of Zinc and Comparison of In Vitro and In Vivo Zinc Uptake of Certain Oil Seed Meals. *Poultry Sci.* 46: 233-241, 1967
3. J. G. Reinhold, A. Parsa, N. Karimian, J. W. Hammick, and F. Ismail-Beigi: Availability of Zinc in Leavened and Unleavened Wholemeal Wheat Breads as Measured by Solubility and Uptake by Rat Intestine In Vitro. *J. Nutrition* 104: 976-982, 1974

NITROSAMINES AND CANCER

A high proportion of rats fed nitrites plus secondary or tertiary amines developed malignant tumors. It is postulated that the nitrosamines produced in vivo as a result of chemical interaction are the causative agents.

Key Words: cancer, nitrites, amines, nitrosamines

It has been known for many years that various environmental hazards are associated with an increased risk of cancer. For example, people who work with creosote and coal tar are more prone to development of cancer of the skin. Since P. N. Magee and J. M. Barnes¹ first showed that nitrosamines caused hepatic cancer in rats there has been considerable interest in these substances.

It is well known that secondary and tertiary amines may react with nitrites in vitro and produce nitrosamines. Since nitrites are widely used in curing food products, it was natural to turn to cured foods and estimate the quantities of nitrosamines found in them. N. T. Crosby et al.² examined several processed foods and found that only very small quantities of the potentially harmful nitroso compounds could be detected. These authors were of course careful to point out that the foods they tested represented a very small fraction of available food products. More recently, N. P. Sen et al.³ examined various

brands of sausages and found that one, made with a specific meat-curing mixture which contained 0.96 percent sodium nitrate and nitrite, was contaminated with nitrosamines. They further established that some spices, such as black pepper and paprika, can react with nitrites to form nitrosamines. The practice of mixing certain spices with nitrites may thus lead to the formation of nitrosamines when the products are stored or further processed.

W. Lijinsky and his colleagues have concentrated more on the possible effects of the formation of nitrosamines in vivo as a result of ingesting nitrites and amines. They showed that rats developed esophageal tumors when they were fed nitrites plus methylbenzylamine.⁴

W. Lijinsky and co-workers⁵ have now reported on some of their experiments in this field. They used three experimental groups of 30 rats each, which were given the following solutions to drink: heptamethyleneimine hydrochloride plus sodium nitrate, aminopyrine at two dose levels (250 mg per liter and 1 g per liter) plus sodium nitrite (the authors first determined

that the prepared mixtures contained negligible quantities of nitrosamines). There were no deaths among the control rats given nitrite, aminopyrine, or heptamethyleneimine hydrochloride alone. Twenty-nine of 30 rats given aminopyrine at the high dose level plus nitrites died with malignant tumors within 30 weeks: 13 of 14 rats who died as a result of taking the lower dose of aminopyrine plus nitrite for 50 weeks were found to have malignant tumors. Heptamethyleneimine hydrochloride plus nitrate produced fatal tumors in 20 of the 23 rats who died after 28 weeks. The animals fed aminopyrine developed mainly hepatic tumors while those on heptamethyleneimine produced pulmonary and esophageal cancers.

The authors make the claim that "the results of these experiments suggest that the interaction of secondary and tertiary amines with nitrites in the stomach may represent an important facet of the etiology of cancer in man." This claim must of course be subject to the usual strictures against transposing the results of animal experiments to the human situation. The authors give no data on the levels of nitrites or amines likely to be ingested by man, and although they state that amines in cigarette smoke may be dissolved in the mouth and swallowed, they give no evidence from their own experience or from the literature that this is a possibility. It has been shown, however,⁶ that nitrosamines may be present in small quantities in condensates of tobacco smoke.

Before this theory is accepted, we need epidemiologic data on human beings that can be related to the laboratory findings. Is there only a dietary reason for the differences in cancer incidence in different parts of the world, or between different groups of people from the same part of the world? If nitrites were eliminated from the diet would the probability of cancer in such high-risk group as heavy cigarette smokers be reduced?

There are other nutritional considerations which must be borne in mind. There is for example, evidence that the effect of dietary carcinogens may be modified by the level of protein intake. With respect to dietary protein, it has been shown that the level of dietary protein and lipotropic factors has a marked effect on the capacity of aflatoxin to produce cancers in rats.^{7,8}

Unfortunately there were no data given in the experiments reviewed here on such things as protein, mineral, and vitamin intake by the various groups of rats relative to each other and relative to the controls. There should be as much interest in those animals which do not develop tumors as in those that do. These and similar questions must flow naturally from this interesting line of research by this group. □

1. P. N. Magee and J. M. Barnes: The Production of Malignant Primary Hepatic Tumours in the Rat by Feeding Dimethylnitrosamines. *Brit. J. Cancer* 10: 114-122, 1956
2. N. T. Crosby, J. K. Foreman, J. F. Palframan, and R. Sawyer: Estimation of Steam-Volatile N-Nitrosamines in Foods at the 1 ug/kg Level. *Nature* (London) 238: 342-343, 1972
3. N. P. Sen, W. F. Miles, B. Donaldson, T. Panalaks, and J. R. Iyengar: Formation of Nitrosamines in a Meat Curing Mixture. *Nature* (London) 245: 104-105, 1973
4. W. Lijinsky and S. S. Epstein: Nitrosamines as Environmental Carcinogens. *Nature* (London) 225: 21-23, 1970
5. W. Lijinsky, H. W. Taylor, C. Snyder, and P. Nettesheim: Malignant Tumors of Liver and Lung in Rats Fed Aminopyrine or Heptamethyleneimine together with Nitrite. *Nature* (London) 244: 176-178, 1973
6. J. W. Rhoades and D. E. Johnson: N-Dimethylnitrosamine in Tobacco Smoke Condensate. *Nature* (London) 236: 307-308, 1972
7. Effects of Aflatoxin on the Liver. *Nutrition Reviews* 27: 121-123, 1969
8. A. E. Rogers and P. M. Newberne: Nutrition and Aflatoxin Carcinogenesis. *Nature* (London) 229: 62-63, 1971

CENTRAL NERVOUS SYSTEM CHANGES IN DEFICIENCY OF VITAMIN B₆ AND OTHER B-COMPLEX VITAMINS

The effects of vitamin B₆ deficiency on the central nervous system are not confined to the critical preweaning period of brain development. Central nervous system abnormalities are associated with deficiencies of thiamin, riboflavin, niacin, folic acid, and biotin, besides pyridoxine.

Key Words: central nervous system, electrophysiological changes, vitamin B₆-deficiency, postweaning period

Nutritional deficiencies cause defects in cellular metabolism of the brain, which lead to electrophysiological changes and finally manifest as behavioral (sensory, motor, intellectual, and personality) abnormalities. Neurodietetic research has primarily relied on behavioral tests based on conditioning and learning experiments, where appetitive behavior or reflex and locomotor responses to electric shock are used as criteria for assessment. Such tests cannot distinguish between effects on appetite and motor system from those on learning. Further, central nervous system (CNS) involvement can only be inferred through behavioral tests.

A more objective and direct assessment of CNS involvement is based on an electrophysiological approach. In recent years electroencephalographic (EEG) techniques of resting tracings, activated tracings, frequency analysis, and evoked response from photic, auditory, and somatosensory stimuli have been used for measuring and monitoring electrophysiological changes in malnutrition.

Detrimental effects of vitamin B₆ deficiency on CNS as reflected in behavioral and electrophysiological changes have been reported in animals and humans.¹ A study in rats suggests that the deficit in avoidance

learning seen in post-weaning vitamin B₆ deficient rats, is distinct from the slight but significant motor deficit suffered by those animals.² M. C. Stephens et al.³ reported that vitamin B₆ deficiency induced in rats during the preweaning period, when active brain development is in progress, can result in the onset of seizures and EEG changes. The deficient animals also show abnormalities in the auditory evoked cortical potentials with respect to their latency, wave form, and response to repetitive stimuli.

In a recent report, C.N. Stewart et al.⁴ showed that the deleterious effects of B₆ deficiency on CNS can be observed even when the deficiency is induced in the postweaning period (from the age of 21 days onwards) when the brain development has been almost completed. Latency of visually evoked cortical response was used as a measure of CNS reaction to stimulation. Chronic electrodes were implanted in the brain with the electrode tips located on the surface of the dura over the visual cortex and vertex. A third electrode which served as ground was placed on the os nasale, to minimize the movement artifact. Each animal was exposed to 50 successive stimuli at five different light intensities. Signals from the visual cortex were amplified and fed into a computer. Averages of 50 stimuli were recorded.

With an increase in intensity of photic stimulation, the latency of response dimin-

ished in control as well as deficient groups. The vitamin B₆ deficient animals showed significantly higher latency at each intensity and this improved after treatment with pyridoxine. These data suggest that the effects of vitamin B₆ deficiency on CNS are not necessarily confined to the critical preweaning period of brain development. The possibility of the observed changes as being due to inanition was ruled out since in an earlier study, these workers had failed to observe the effect of food restriction on cortical evoked responses.⁴ Besides, the CNS abnormalities could be reversed by the administration of pyridoxine.

The neurochemical basis of CNS lesions in vitamin B₆ deficiency is not understood. Induction of vitamin B₆ deficiency in the preweaning stage reduces the DNA, RNA, and protein content of the brain, but similar changes are not observed when the deficiency is induced in the postweaning stage.⁵ The levels of serotonin and noradrenaline are not altered in vitamin B₆ deficiency.⁶ The depletion of pyridoxal phosphate (PLP) in the brain of rats fed on vitamin B₆ deficient diet is 50 percent, and not as high as that in other tissues.⁴ The activities of some PLP enzymes in the brain fall³ and the synthesis of gamma-aminobutyric acid in the brain decreases in B₆ deficiency.^{3,7} The latter may lead to increased CNS excitability.

Behavioral and/or electrophysiological abnormalities were also observed in the deficiencies of other B-complex vitamins such as thiamin,⁸ riboflavin,^{9,10} niacin,¹¹ folic acid,^{12,13} and biotin.¹⁴ These changes in some instances were found to precede other clinical manifestations of the deficiency syndrome.^{8,10} Some early literature on the effects of B-complex vitamin deficiencies on brain function has been reviewed by J. Brozek and G. Vaes.¹⁵ Much of the older data is difficult to evaluate since experiments were carried out with only semi-pure diets. The recent studies, however, leave little doubt that the deficiency of many B-complex vitamins in early or adult life can have profound effects on the CNS. The implications and

consequences of such abnormalities in brain function on the overall performance of populations where widespread malnutrition exists need attention and investigation. □

1. D. B. Coursin: Vitamin B₆ and Brain Function in Animals and Man. *Ann. N. Y. Acad. Sci.* 166: 7-15, 1969
2. C. N. Stewart, H. N. Bhagavan, and D. B. Coursin: Some Behavioral Consequences of Pyridoxine Deficiency in Rats in *International Symposium on Pyridoxal Enzymes*. L. Yamada, N. Katunuma, and H. Wada, Editors, p. 181-183, Maruzen Co., Tokyo, 1968
3. M. C. Stephens, V. Havlicek, and K. Dakshinamurti: Pyridoxine Deficiency and Development of the Central Nervous System in the Rat. *J. Neurochem.* 18: 2407-2416, 1971
4. C. N. Stewart, D. B. Coursin, and H. N. Bhagavan: Cortical-Evoked Responses in Pyridoxine-Deficient Rats. *J. Nutrition* 103: 462-467, 1973
5. H. N. Bhagavan and D. B. Coursin: Effect of Pyridoxine Deficiency on Nucleic Acid and Protein Contents of Brain and Liver in Rats. *Int. J. Vit. Nutrition Res.* 41: 419-423, 1971
6. H. N. Bhagavan and D. B. Coursin: Effects of Pyridoxine Deficiency and DL-p-Chlorophenylalanine Administration to Rats on 5-Hydroxytryptamine Concentrations in Brain and 5-Hydroxytryptamine Concentrations in Blood. *Biochem. J.* 134: 763-767, 1973
7. J. K. Tews and R. A. Lowell: The Effect of Nutritional Pyridoxine Deficiency on Free Amino Acids and Related Substances in Mouse Brain. *J. Neurochem.* 14: 1-7, 1967
8. M. R. Peskin, G. Newton, and M. Brin: Thiamine Deficiency, Infantile Manipulation and Startle Response in Rats. *J. Nutrition* 20-24, 1967
9. T. Arakawa, T. Mizuno, F. Chiba, K. Sakai, S. Watanabe, T. Tamura, S. Tatsumi, and D. B. Coursin: Frequency Analysis of Electroencephalograms and Latency of Photically Induced Average Evoked Responses in Children with Ariboflavinosis. (Preliminary Report) *Tohoku J. Exp. Med.* 94: 327-335, 1968
10. R. T. Sterner and W. R. Price: Restricted Riboflavin: Within-Subject Behavioral Effects in Humans. *Am. J. Clin. Nutrition* 26: 150-160, 1973
11. S. G. Srikantia, M. V. Reddy, and K. Krishnaswamy: Electroencephalographic Patterns in

- Pellagra. *Electroenceph. Clin. Neurophysiol.* 25: 386-388, 1968
12. T. Arakawa, T. Mizuno, Y. Honda, T. Tamura, J. Sakai, S. Tatsumi, F. Chiba, and D. B. Coursin: Brain Function of Infants Fed on Milk from Mothers with Low Serum Folate Levels. *Tohoku J. Exp. Med.* 97: 391-397, 1969
 13. R. A. Schreiber and J. W. Zempi: Neonatal Folate Deficiency: Effect on Audiogenic Seizures in DBA/2J Mice. *Nutrition Rep. Int.* 8: 237-244, 1973
 14. C. N. Stewart, H. N. Bhagavan, D. B. Coursin, and K. Dahshinamurthi: Effect of Biotin Deficiency on Escape and Avoidance Learning in Rats. *J. Nutrition* 88: 427-433, 1966
 15. J. Brozek and G. Vaes: Experimental Investigations on the Effects of Dietary Deficiencies on Animal and Human Behavior. *Vitamins Hormones* 19: 43-94, 1961

COMPETITION BETWEEN PHLORIZIN AND GOLD THIOGLUCOSE FOR GLUCORECEPTOR CELL TRANSPORT MECHANISMS IN THE HYPOTHALAMUS

Phlorizin injected locally in the region of the medial hypothalamus protects glucoreceptor cells from necrosis due to gold thioglucose injections and defines a group of cells which are particularly associated with the regulation of food intake in mice.

Key Words: glucose transport, food intake, hypothalamus, phlorizin

The proposed mechanism by which glucoreceptor cells of the hypothalamus regulate food intake depends on the uptake of glucose by the cells through specific transporting processes. Some substances which inhibit or compete for carrier mechanisms involved with glucose uptake in other cells of the body have been investigated as they relate both to changes in glucose transport in the glucoreceptor cell and to changes in food intake.

Phlorizin inhibits glucose transport by the enterocyte of the gut and if phlorizin inhibits glucose transport in these cells, then the cells should not respond to high blood glucose concentrations and fail to shut off food intake. When phlorizin was injected locally into the lateral cerebral ventricle of the rat, a state of hyperphagia and weight gain was produced.¹ Similar amounts given intraperitoneally failed to produce effects on food intake. A hypothesis was developed that local instilla-

tion of phlorizin inhibited the adjustment of food intake according to caloric expenditure as it relates to glucose concentration in blood by the inhibition of glucose transporting mechanisms.

A. F. Debons and associates² further extended the test of this hypothesis by injecting phlorizin intrahypothalamically. They followed this injection with gold thioglucose which, as has been shown in the past, destroys glucoreceptor cells. Phlorizin was labeled with radioactive hydrogen so that autoradiographic localization of phlorizin in brain sections could be done to define the region of phlorizin inhibition in the hypothalamus.

Phlorizin protected 16 of 18 mice studied from the usual necrosis of the ventromedial area of the hypothalamus produced after gold thioglucose injection. In 13 of the animals, prevention was unilateral, on the side of the injection; in the remaining three, prevention was bilateral. These data suggested a local action of phlorizin which was enlarged due to diffusion across tissue in the region of the

third ventricle. In contrast to these studies, when saline was injected, necrosis of the ventromedial area was found in 15 of 17 mice. Phlorizin was effective if injected within a distance of approximately 1 mm from the midline of the brain.

Autoradiography of four animals revealed heavy labeling of cells in the ventromedial hypothalamus. These cells were interspersed among a large number of unlabeled cells. The labeled cells were most heavily concentrated in the lateral part of the arcuate nucleus and the area between the arcuate and ventromedial nuclei. Labeled cells extended in a band laterally along the ventral border of the hypothalamus for a distance of about 1 mm (about halfway to the lateral border of the hypothalamus). The cells in the rostrocaudal direction were labeled for approximately the middle third of the hypothalamus. The area of distribution of phlorizin labeled cells coincided with areas previously described in which necrosis and desolution of cells first appear after gold thioglucose administration.

The cells labeled with phlorizin resembled oligodendrocytes in that they were about the same size, smoothly rounded or oval, with dense membranes, and abundant chromatin. They differed from the oligodendrocyte nuclei in having more finely divided chromatin and the prominent nucleolus. The readily visible "halo" of the perinuclear cytoplasm, characteristic of the oligodendrocytes, was not seen in the phlorizin-labeled cells. Within the cell labeled with phlorizin the most concentrated portion was within the perinuclear region. The nuclei were free of phlorizin. The cells occurred in groups of two or three contiguous cells.

The data supported the hypothesis that hyperphagia induced by intraventricular injection of phlorizin was most likely mediated by the inhibitory action of phlorizin on glucose transport of glucoreceptor cells of the ventromedial hypothalamus. Phlorizin acted on the ventromedial region of the hypothalamus and was effective in preventing necrosis usually

occurring after gold thioglucose injection. Most likely, it is the glucose transporting mechanism that is responsible for absorption of gold thioglucose into these nervous cells which then induces necrosis and phlorizin prevented the uptake of gold thioglucose. Further, phlorizin appearing in a particular single type of cell in a cellularly heterogenous area, further supported the concept of a specific glucoreceptor cell which regulates feeding behavior.

Previous data, on the other hand, have been advanced that gold thioglucose produces inflammatory changes due to toxic reactions within capillary walls rather than affecting a particular glucoreceptor cell.³

It has been shown that the glucose analogue, 2-deoxyglucose, prevents gold thioglucose-induced obesity as well, most likely by competing for transport mechanisms as does phlorizin within the glucoreceptor cells.⁴ When 2-deoxyglucose is given alone, it induces pronounced hyperphagia as does phlorizin. Further implications of glucose transporting mechanisms is that insulin deficiency prevents gold thioglucose-induced necrosis of the ventromedial hypothalamus while administration of insulin rapidly restores the sensitivity of gold thioglucose.⁵ Finally, the binding of phlorizin by cells which seem to be the same cells that undergo necrosis due to gold thioglucose, suggests there are specific cells sensitive to gold thioglucose.

These interesting data combined with previous reports of the interaction between phlorizin, deoxyglucose, insulin, and gold thioglucose on a specific cell in the ventromedial hypothalamus suggest several additional experiments which would be of much interest in better understanding the regulation of food intake by glucoreceptor cells. For instance, ouabain inhibits the active transporting mechanism of sodium and in turn, inhibits the active transporting mechanism of glucose in the gut. If active transport of sodium is a requirement for glucose transport by the glucoreceptor cells then injection of ouabain into the hypothalamus should produce hyperphagia.

These studies suggest possibilities which might explain the inability to clearly apply the glucoreceptor theory to explain food intake in human beings. For instance, because of the many naturally occurring compounds which compete for carriers involved with glucose transport, transport of glucose into the glucoreceptor cell could be delayed and the reflex "damped" for turning off food intake. Some amino acids compete for these carrier mechanisms and are usually components of absorbed nutrients along with glucose. If amino acids partially tie up the carrier mechanisms in the glucoreceptors, the response to high blood glucose would not be as prompt as they would be in the absence of amino acids.

These studies may offer some hope for better pharmacologic control of appetite. For instance, if phlorizin proves nontoxic for human beings and if it does not permanently damage tissues by tying up transporting mechanisms for glucose, then such a drug might have a role in the treatment of anorexia nervosa.

Similar studies on the induction of "satiety" might be helpful. The treatment of obesity and hyperphagia is disappointing

at present in the human being; none of the physiological experiments which deal with appetite control in animals has been of much use in preventing or treating obesity and inhibiting food intake. The identification of substances which facilitate or inhibit glucose transport into glucoreceptor cells of the hypothalamus may be a promising area of investigation. □

1. Z. Glick and J. Mayer: Hyperphagia Caused by Cerebral Ventricular Infusion of Phlorizin. *Nature* (London) 219: 1374, 1968
2. A. F. Debons, I. Krimsky, A. From, and H. Pattinian: Phlorizin Inhibition of Hypothalamic Necrosis Induced by Gold Thioglucose. *Am. J. Physiol.* 226: 574-578, 1974
3. Z. E. Caffyn: Early Vascular Changes in the Brain of the Mouse after Injection of Gold Thioglucose and Bipiperidyl Mustard. *J. Path.* 106: 49-56, 1972
4. H. J. Likuski, A. F. Debons, and R. J. Cloutier: Inhibition of Gold Thioglucose-Induced Hypothalamic Obesity by Glucose Analogues. *Am. J. Physiol.* 212: 669-676, 1967
5. A. F. Debons, I. Krimsky, A. From, and R. J. Cloutier: Rapid Effects of Insulin on the Hypothalamic Satiety Center. *Am. J. Physiol.* 217: 1114-1118, 1969

VITAMIN K AND THE CARBOXYLATION OF GLUTAMYL RESIDUES IN THE FORMATION OF PROTHROMBIN

Evidence is presented for the occurrence of a new amino acid, gamma-carboxyglutamic acid in prothrombin. It is proposed that this amino acid accounts for the calcium-binding properties of prothrombin. A role for vitamin K in the carboxylation of protein gamma-glutamyl residues is indicated.

Key Words: vitamin K, prothrombin, gamma-glutamyl carboxylation, gamma-carboxyglutamic acid

The formation of prothrombin is a vitamin K dependent process. In animals deficient in vitamin K or receiving coumarin anticoagulants, a protein is formed which has chemical and immunological properties similar to prothrombin. This protein, desig-

nated abnormal prothrombin, can be converted to thrombin by in vitro procedures but it is not so converted in vivo. Abnormal prothrombin differs mainly from prothrombin in its inability to bind calcium, a process essential to the in vivo conversion of prothrombin to thrombin and initiation of the blood clotting mechanism. Hence, interest^{1,2} has focused on the nature of the

groupings in prothrombin which bind calcium because their formation would seem to be intimately bound to the mode of action of vitamin K. Evidence has now been presented by two different laboratories that the calcium-binding properties of prothrombin are probably due to its content of a new amino acid, gamma carboxyglutamic acid. Vitamin K thus appears to function by inducing the carboxylation of glutamyl residues in abnormal prothrombin and thereby converting it to prothrombin.

In the studies of J. Stenflo and co-workers³ attention has focused on a comparison of the properties of peptides isolated from the NH₂-terminal portions of both prothrombin and abnormal prothrombin. It has been previously shown that it is in this portion of the prothrombin molecule that its calcium-binding groups are localized. Employing BrCN and trypsin, a heptapeptide was isolated from prothrombin which had a higher anodal electrophoretic mobility at a pH of 6.5 than the corresponding heptapeptide obtained from abnormal prothrombin. Further digestion of this heptapeptide with aminopeptidase and carboxypeptidase yielded a tetrapeptide that still has an abnormally high anodal electrophoretic mobility at a pH of 6.5. Acid hydrolysis of this tetrapeptide yielded two molecules of glutamic acid and one each of valine and leucine. From the known sequence of the heptapeptide (unpublished data) the authors were able to conclude that the tetrapeptide had the basic structure Leu-Glu-Glu-Val. This tetrapeptide was therefore synthesized and it was found to have an electrophoretic mobility lower than that of the tetrapeptide isolated from prothrombin. The native and the synthetic tetrapeptide were both subjected to ¹H nuclear magnetic resonance spectroscopy. The spectrum of the native peptide showed no resonances not shown by the synthetic peptide but did indicate that the native peptide had lost approximately four more protons during the deuterium exchange process than the synthetic peptide. The region of the spec-

trum in which this loss of protons occurred was that which could be assigned to the gamma-carbon of the glutamyl residue of the synthetic peptide. Mass spectrometry of the N-acetylated and methyl esterified tetrapeptides was then performed. These results combined with those from the NMR study were consistent with the conclusion that in the native tetrapeptide an extra carboxyl group was attached to the gamma-carbon atom of the glutamyl residues of the synthetic peptide. The structure of the native peptide was therefore deduced to be Leu-Glx-Glx-Val where Glx represents the residue of a new amino acid, gamma-carboxyglutamic acid (3 amino-1, 1, 3 propane tricarboxylic acid). When the native peptide is briefly heated at 150° in its acid form, decarboxylation occurs and the electrophoretic mobility of the product becomes identical with that of the synthetic peptide.

The other investigation in which evidence is presented for the carboxylation of a glutamyl residue by the action of vitamin K is by J-M. Girardot et al.⁴ The impetus to this study came from the development in B. C. Johnson's laboratory of an in vitro procedure for the formation of clotting factor X using a cell-free liver preparation. Synthesis of factor X in this system is absolutely dependent upon the vitamin K status of the animal, and bicarbonate was found to be a required ingredient of the incubation medium. This suggested that vitamin K might be involved in a CO₂ fixation reaction. In order to test this hypothesis, the possibility that radioactive CO₂ could be incorporated into prothrombin in vivo was investigated. Two groups of rats were employed; one group received warfarin approximately 20 hours prior to the experiment. On the day of the experiment both groups were given intraperitoneal injections of vitamin K₁ and of ¹⁴C-sodium bicarbonate. After two hours the experiment was terminated and prothrombin isolated from the rat plasma by BaSO₄ adsorption and further purified by DEAE-Sephadex chromatography. Radioactivity was found in the pro-

thrombin from both groups of rats, though that from the warfarin treated rats showed three to four times as much ^{14}C incorporation per milligram of protein as the control group.

The labeled prothrombin was digested with trypsin, the digest treated with Fluram solution and the mixture of peptides separated by disc gel electrophoresis. One peptide, which migrated toward the anode ahead of all the others and in advance of the bromophenol blue used as a marker, contained all the radioactivity. Digestion of the labeled prothrombin was also carried out with pronase and aminopeptidase. Paper chromatography of this digestion mixture in a water saturated phenol system revealed an amino acid containing radioactivity which ran ahead of both aspartate and glutamate. On the amino acid analyzer the labeled amino acid appears in the cysteic acid fraction. Acid digestion of this radioactive fraction eliminates the radioactivity and an unlabeled amino acid appears at the glutamic acid position in the amino acid analyzer. The authors conclude that "the data presented indicate that the vitamin K-dependent step in the formation of prothrombin is the carboxylation of a glutamyl residue of a preformed protein precursor."

Both of the reports dealt with here are preliminary in nature and hence leave some questions unanswered. Taken together, however, they provide strong evidence for the existence of a new amino acid in prothrombin. This amino acid would appear to be gamma-carboxyglutamic acid. Nevertheless, the isolation of this amino acid in sufficient amounts to permit further characterization will be welcome. From the standpoint of providing calcium-binding properties to a protein, the incorporation of an amino acid such as gamma-carboxyglutamic acid makes good sense. The work of Stenflo et al. indicates that two residues of the new amino acid adjoin one another in the tetrapeptide they isolated from prothrombin. Two adjoining residues of gamma-carboxyglutamic acid would provide four carboxyl groups in

close proximity, a situation reminiscent of such commercial calcium chelators as Versene. The exact number of the new amino acid residues in prothrombin and whether they always occur in paired sequence remains to be determined. Stenflo et al. state that the new amino acid is also present in a larger peptide, containing approximately 22 amino acid residues, that has been isolated from prothrombin. It is interesting to note that eight of the 13 amino acids formed by the acid hydrolysis of the peptide isolated from prothrombin by G. L. Nelsestuen and J. W. Suttie² were glutamic acid. Since this peptide was reported by them to bind about three moles of calcium per mole, it will be of interest to know how many of the eight glutamic acids were produced by decarboxylation of the new amino acid during hydrolysis of the peptide. Evidence is cited by both Stenflo et al. and Girardot et al. that this new amino acid is probably present in other calcium-binding proteins that are involved as factors in the clotting mechanism. Thus, workers interested in other areas where protein binding of calcium or other metals is involved, may well give heed to the occurrence of this new amino acid.

The most exciting aspect of these new findings is the clue that it affords to the mode of action of vitamin K. The involvement of this vitamin in what appears to be a carboxylation reaction raises many interesting questions. Does vitamin K participate directly in this reaction or is it involved in the production of an enzyme which carries out this reaction? Does biotin, which is involved in the carboxylation of numerous small molecules, play a role in the process? Answers to these and many other questions will now be sought. A giant step forward in our understanding of the action of vitamin K has been provided by the two groups of workers whose studies have been reported here.

Addendum

Since completion of this review a report has appeared describing the isolation from

a calcium-binding fragment of prothrombin of the dipeptide gamma-carboxylglutamylserine. Identification of the new amino acid in this dipeptide was made by means of mass spectrometry.⁵ □

1. Vitamin K and Prothrombin Structure. *Nutrition Reviews* 32: 279-281, 1974
2. G. L. Nelstuen and J. W. Suttie: The Mode of Action of Vitamin K. Isolation of a Peptide Containing the Vitamin K-Dependent Portion of Prothrombin. *Proc. Nat. Acad. Sci. USA* 70: 3366-3370, 1973
3. J. Stenflo, P. Fernlund, W. Egan, and P. Roepstorff: Vitamin K Dependent Modifications of Glutamic Acid Residues in Prothrombin. *Proc. Nat. Acad. Sci. USA* 71: 2730-2733, 1974
4. J-M. Girardot, R. Delaney, and B. C. Johnson: Carboxylation, the Completion Step in Prothrombin Biosynthesis. *Biochem. Biophys. Res. Commun.* 59: 1197-1203, 1974
5. G. L. Nelstuen, T. H. Zykovicz, and J. B. Howard: The Mode of Action of Vitamin K. Identification of gamma-carboxylglutamic acid as a component of prothrombin. *J. Biol. Chem.* 249: 6347-6350, 1974

Nutrition Reviews

authoritative articles by distinguished international scientists • competent, critical, and knowledgeable reviews • interpretative summaries of important original clinical and experimental research • selected excerpts from classic publications marking milestones in nutrition research • informative special reports on government and organization actions • contributing editors review journals of widespread interest covering biochemistry, food science, public health, toxicology, dentistry, pediatrics, internal medicine, gastroenterology and other clinical specialities •

Send subscription and renewal requests to:

THE NUTRITION FOUNDATION
489 Fifth Avenue
New York, New York 10017

\$12.00 per year

\$6.00 for students

Termination of AMA Council on Foods and Nutrition

The AMA Board of Trustees has terminated the Council on Foods and Nutrition after 46 years of service to the medical profession. Born when vitamins were little more than medical curiosities, the Council has dealt with increasingly complex issues, terminating when man has learned how to nourish with chemically defined diets.

The multifarious Council, working to promote the nutritional health of the nation, to support the development of clinical nutrition, to encourage nutrition awareness in the food industry, and to develop concepts in food safety and regulation, had achieved world renown as a mold of opinion and conscience. Nearly every institution has felt the impact of the Council. Statements of the Council and its review articles were used in many ways — from use in elementary educational concepts — to the formulation of governmental policy. Its symposia, conferences and congresses served to coalesce knowledge and stimulate needed research. Concerns of the Council dealt with matters varying from vending machines in schools to proposals for new concepts in the regulation of food quality and safety and proposals for improving the nutritional value of a nation's food supply.

The Council fostered the logical development of therapeutic nutrition. It provided guidelines for the use of vitamin and mineral preparations and helped to frame regulations that would ensure the proper formulation of dietary supplements. The Council on Foods and Nutrition worked diligently to provide means whereby every person could be assured an adequate intake of nutrients by its fostering of regulations

and standards for enriched and fortified foods. It provided textual materials for education and led the way in encouraging the teaching of nutrition in undergraduate and postgraduate medical education. The Council identified with over 350 colleges and universities in a program to improve appreciation of the medical sciences and to tell of a small aspect of the science of nutrition. More than 140 medical students were introduced to the methods of scientific research by a program of research fellowships.

The Council on Foods and Nutrition testified before the Congress of the United States on such issues as the nutritional quality of the U.S. food supply, nutritional fraud directed to the elderly, the need for support in nutrition education of the profession and the public, the quackery in weight reduction schemes, the need for regulations controlling the proper use of vitamin preparations and dietary supplements, and of the need for national policies on food and nutrition.

The Council worked easily with all segments of the food and the pharmaceutical industries, with various branches of state and federal governments, with the many medical and nutrition societies and organizations, with institutions of higher education, with food trade associations, and with individuals. The Council molded opinions and brought harmony from diversity.

With more than 100 publications, including over a dozen books, the Council made its mark and became an institution. That institution is now dead but its good works are recorded for posterity. Some of its works now gather dust but others lead

the way for the emergence of new and exciting concepts in human welfare. The Council was ubiquitous, it is now an archive.

Those of you who have served the American Medical Association through its Council on Foods and Nutrition must know the sadness that I feel with the knowledge that an institution has passed. Please know that I am immensely proud to have been associated with each of you as your Council Secretary. Our triumphs lifted me; our failures depressed me.

This note closes the official Bulletin of the Council and will reside with past Bulletins in the Archive Library of the American Medical Association.

Philip L. White, Sc.D.

Meeting Announcements

The Council on Epidemiology and Prevention of the International Society of Cardiology will hold its Eighth Ten Day International Teaching Seminar on Cardiovascular Epidemiology in Mexico, September 21 through October 3, 1975.

For further information contact:
Jeremiah Stamler, M.D., Secretary
Council on Epidemiology and Prevention,
I.S.C.

c/o Northwestern University Medical
School

Ward Building — Room 9-105
303 East Chicago Avenue
Chicago, Illinois 60611

A short course, entitled, "Nutrition in Mothers, Infants, and Pre-school Children", will be held at the Carolina Inn, Chapel Hill, March 3-4, 1975. It will be sponsored by the Department of Nutrition, University of North Carolina at Chapel Hill. For further information contact Dr. John J. B. Anderson, Department of Nutrition, School of Public Health, UNC-Chapel Hill, Chapel Hill, N.C. 27514.

FTC Proposed Trade Regulation Rule on Food Advertising

A proposed trade regulation rule designed to cover voluntary nutrition claims made in food advertising was announced by the Federal Trade Commission on November 7. The FTC also published a staff paper recommending mandatory disclosures of certain nutrition information in all food advertisements.

The following categories of nutrition claims are covered:

Emphatic Nutrition Claims which refer to the amounts of various vitamins and minerals and protein in a food (e.g., "Loaded with Vitamin A").

Nutrient Comparison Claims concerning specific nutrients in compared foods and with respect to the comparative nutritional values of foods (e.g., "Food X has more Vitamin A than Food Y").

Nourishment Claims relating to the overall nutritional values of foods (e.g., "Food X is nutritious").

Claims for Foods Intended to be Combined with Other Foods (e.g., "Food X, when combined with Food Y, gives you nourishing Vitamin A").

The proposed rule is accompanied by an Explanation of Proceeding and Analysis and Statement of Issues by Section which describes the various provisions of the proposed rule and sets forth issues pertaining to certain of those provisions. The Commission desires comments addressed specifically to those issues. This document also invites comment on issues relating to the subjects of Natural and Organic Food Claims; Fat, Fatty Acid and Cholesterol Content Claims; and Health and Related Claims.

Copies of the proposed rule and accompanying documents are available upon request to Legal and Public Records, Federal Trade Commission, Washington, D. C. 20580. Persons wishing to file written comments on the proposed rule should address them to the Special Assistant Director for Rulemaking at the above address by February 5, 1975.

Letter to the Editor

Stereochemical Orientation of 1,25-(OH)₂-D₃

Sir: I am writing to point out an important error in the article communicated by M. R. Haussler which appeared in *Nutrition Reviews* 32:257, 1974. He states on page 259 that "recently, Semmler et al. and Barton et al. have chemically synthesized 1 α ,25-dihydroxyvitamin-D₃ (1 α ,25-(OH)₂-D₃) as well as 1 α -OH-D₃ to prove unequivocally that the hydroxyl on carbon number 1 of the natural hormone has an α -stereochemical orientation". This is only circumstantial evidence and not unequivocal proof. The natural material, 1,25(OH)₂-D₃, has not been crystallized nor has the configuration of the hydroxyl group at position 1 been determined. Until this unequivocal proof of structure is obtained, one should be cautious in interpretation.

The data, which Haussler cites, show that synthetic analogs of vitamin D metabolites, with an α -stereochemical orientation at position 1, have a biopotency similar to the natural hormone. Until one shows that the biopotency of synthetic 1- β isomers is different from the 1- α isomers, the evidence is presumptive at best.

It may well be that the 1- α assignment will prove correct, but this can be established only by direct experimentation, not conjecture or extrapolation.

Howard Rasmussen, M.D., Ph.D.
*Benjamin Rush Professor of
Biochemistry, and
Professor of Medicine
University of Pennsylvania*

Recent Books

National Institute of Nutrition, Annual Report, January 1, 1973 to December 31, 1973. Published by the Indian Council of Medical Research, Hyderabad, 500 007, India. Pp. 170.

Laboratory Tests for the Assessment of Nutritional Status, by H. E. Sauberlich, R. P. Dowdy, and J. H. Skala. Published by CRC Press, Inc., Cleveland, Ohio. Price \$9.95.

Total Parenteral Nutrition, edited by P. L. White and M. E. Nagy. Published by Publishing Sciences Group, Inc., Acton, Massachusetts. Pp. 477. Price, \$24.

The Milk-Free and Milk-Free, Egg-Free Cookbook, by I. S. Sainsbury. Published by Charles C. Thomas, Publisher, Springfield, Illinois, Pp. 148. Price \$8.95.

The Meaning of Human Nutrition by M.W. Lamb and M.L. Harden. Published by Pergamon Press, Inc., Maxwell House, Fairview Park, Elmsford, New York 10523. Pp. 284. Price \$11.50 (hardcover), \$6.50 (softcover).

Nutrients in Processed Foods. Vitamins and Minerals, by H. E. Bauman et al. Published by Publishing Sciences Group, Inc., 411 Massachusetts Avenue, Acton, Massachusetts 01720. Pp. 193. Price \$16.00.

Nutrition and Anti-Infectious Defense by Iancu Gontzea, 2nd edition. Published by S. Karger, P.O. Box 352, White Plains, New York 10602. Pp. 287. Price \$19.00.

Safe Central Venous Nutrition: Guidelines for Prevention and Management of Complications, by Mohamad. H. Parsa, Jose M. Ferrer, and David V. Habif. Published by Charles C. Thomas, Publisher, Springfield, Illinois. Pp. 266. Price \$17.50.

Aromatic Amino Acids in the Brain, Ciba Foundation Symposium 22. Published by American Elsevier Publishing Company, Inc., 52 Vanderbilt Avenue, New York, New York 10017. Pp. 396. Price \$23.50.

Dietary Fats and Thrombosis, Proceedings of the INSERM Symposium, S. Renaud and A. Nordoy, editors. Published by S. Karger AG, Arnold-Bocklin-Strasse 25, CH-4011 Basel, Switzerland. Pp. 180. Price \$26.05.

The Control of Metabolism, edited by J. S. Sink. Published by The Pennsylvania State University Press. Pp. 267. Price \$15.00.

Infant Nutrition, by S.J. Fomon. Published by W.B. Saunders Company, West Washington Square, Philadelphia, Pennsylvania 19105. Pp. 575.

Early Malnutrition and Mental Development, Symposia of the Swedish Nutrition Foundation XII, edited by J. Cravioto, L. Hambræus, and B. Vahlquist. Published by Almqvist & Wiksell, Uppsala, Sweden. Pp. 331. Price Sw. kr. 75.

World Review of Nutrition and Dietetics, vol. 19, edited by G.H. Bourne. Published by S. Karger, P.O. Box 352, White Plains, New York 10602. Pp. 319. Price \$60.25.

Nutritional Assessment in Health Programs, edited by G. Christakis. Published by the American Public Health Association, Washington, D. C. Pp. 90. Price \$4.00.

Food and Nutrition (1945-1972): Annotated Bibliography; Author and Subject Index. Published by Unipub, New York, New York Pp. 540. Price \$6.00

The following books are available from the National Academy of Sciences, Printing and Publishing Office, 2101 Constitution Avenue, Washington, D. C. 20418:

Orientations in Geochemistry. Publication No. ISBN 0-309-02147-2. Pp. 122. Price \$5.75.

Geochemistry and the Environment: Volume 1, The Relation of Selected Trace Elements to Health and Disease. Publication No. ISBN 0-309-02223-1. Pp. 113. Price \$8.50

Manganese. Publication No. ISBN 0-309-02143-X. Pp. 192. Price \$6.25.

Chromium. Publication No. ISBN 0-309-02217-7. Pp. 155. Price \$6.50.

Vanadium. Publication No. ISBN 0-309-02218-5. Pp. 117. Price \$5.25.

Science and Technology in Presidential Policymaking. A Proposal. Report of the Committee on Science and Technology. Pp. 56.

New Publication

The Baroda Journal of Nutrition is published twice a year by the Biochemistry Department of the Baroda University. The journal is comprehensive in scope, including papers and reviews concerned with varied aspects of nutrition. Subscription rates are \$5 for libraries and institutions, \$3 for individual subscribers, and \$2 for students. Subscriptions are payable in advance to The Baroda Journal of Nutrition, Biochemistry Department, Baroda University, Baroda, India.

The Small Intestine in Vitamin B₁₂ and Folate Deficiency

20 APR 1975

by Charles H. Halsted, M.D.



Vitamin B₁₂ and folate play interrelated roles in nucleoprotein synthesis. Deficiency of each vitamin is expressed by the same morphologic abnormality of the hematopoietic system, a megaloblastic bone marrow. Since the renewal time of the small intestinal epithelium is extremely rapid (two to five days) one would expect a requirement for vitamin B₁₂ and folate for mucosal nucleoprotein synthesis in order to maintain villus anatomy and the functional integrity of the mucosal cell. Thus, it has been postulated that "a nutrient deficiency, be it nutritional or otherwise, may have a deleterious effect on intestinal absorption in general or on the absorption of the nutrient in particular".¹ During the past decade a number of clinical observations have supported the concept that vitamin B₁₂ or folate deficiency may interfere with processes of intestinal absorption. V. Herbert has estimated that as many as 40 percent of patients with pernicious

anemia may be misdiagnosed as having primary intestinal disease on the basis of the second stage Schilling test.² The Schilling test as commonly performed requires two stages. In the first stage ⁵⁷Co labeled vitamin B₁₂ is administered alone and urine collected for 48 hours following a "flushing dose" of 1 mg parenteral vitamin B₁₂. Low excretion could be the result of a deficiency of an intrinsic factor in the stomach, or of intestinal disease including conditions leading to bacterial overgrowth, pancreatic insufficiency, or disease or resection of the terminal ileum.³ Deficiency of intrinsic factor as a cause of vitamin B₁₂ malabsorption is conventionally demonstrated in the second stage Schilling test by a normal urinary excretion of ⁵⁷Co following administration of labeled vitamin B₁₂ together with an exogenous source of intrinsic factor. However, as described below, a significant number of patients with pernicious anemia have abnormal second stage Schilling tests. Precise diagnosis of pernicious anemia therefore requires demonstration of histamine-fast achlorhydria and lack of intrinsic factor in the gastric juice in a patient shown to have low circulating levels of vitamin B₁₂ with megaloblastic anemia.²

R. Carmel and V. Herbert described ten patients with pernicious anemia (low circulating vitamin B₁₂, absence of gastric

Dr. Halsted is Assistant Professor of Internal Medicine, Department of Internal Medicine, School of Medicine, University of California, Davis, California 95616.

limited number of reprints of this article may be obtained from the author. THERE ARE NO REPRINTS OF UNSIGNED REVIEWS.

intrinsic factor with achlorhydria).⁴ The first stage Schilling test was abnormal in all ten and in four the urinary excretion of ⁵⁷Co remained low following the second stage Schilling test. Intestinal absorption was further evaluated in these four patients, all of whom had slightly abnormal absorption of D-xylose. These abnormalities returned to normal one to ten months later with vitamin B₁₂ therapy, suggesting that vitamin B₁₂ deficiency had indeed affected intestinal mucosal function.

J. Lindenbaum et al. recently reported a more extensive study of intestinal absorption in patients with pernicious anemia.⁵ In this series of 28 patients, all had megaloblastic bone marrows, low circulating levels of vitamin B₁₂ with high levels of serum folate, and histamine-fast achlorhydria. Blocking antibodies to intrinsic factor were found in the serum of 17 patients. Intestinal absorption was assessed prior to the receipt of vitamin B₁₂, and consisted of the D-xylose absorption test, measurement of fecal fat excretion, and the standard Stage II Schilling test. By reversing the order of the stages of the Schilling test, the authors were able to measure the absorption of the vitamin B₁₂-intrinsic factor complex prior to the administration of pharmacologic amounts of parenteral vitamin B₁₂ conventionally given as a "flushing" procedure with the Stage I test. Abnormal intestinal absorption of D-xylose was found in 29 percent, of dietary fat in 9 percent, and of the vitamin B₁₂-intrinsic factor complex in 75 percent of the patients studied. Intestinal malabsorption did not correlate with the severity of the megaloblastic anemia. In all but four patients normal Schilling tests were demonstrated following treatment periods with vitamin B₁₂ which ranged from 21 to 100 days. Two of the four refractory patients responded following a five day course of the intestinal antibiotic tetracycline. This study thus lends strong support to the concept that the small intestinal mucosa possesses a metabolic requirement for vitamin B₁₂. Since ileal transport, as measured by the Schilling test, was more

severely affected than jejunal processes, measured by the D-xylose absorption test, the data suggested that the ileum is more significantly affected by vitamin B₁₂ deficiency.

Alternatively, intraluminal bacterial overgrowth in pernicious anemia could compete with ileal mucosa for the vitamin B₁₂-intrinsic factor complex. Bacterial overgrowth of the small intestine is frequent in achlorhydric patients, since gastric acid is a natural barrier to the survival of ingested organisms. W. Sherwood et al. described nine patients with pernicious anemia in whom significant numbers of bacteria (10⁵ to 10⁸ organisms per milliliter) could be cultured from the duodenal contents.⁶ However, there was no change in bacterial counts following vitamin B₁₂ therapy, and only minimal in vitro bacterial binding of vitamin B₁₂-intrinsic factor could be demonstrated. Thus, the bacterial overgrowth was a reflection of achlorhydria but not a cause of vitamin B₁₂ malabsorption. A definitive study of the bacterial contents of the ileal lumen in pernicious anemia has not yet been performed. Such a study might have more significance since the ileum is the site of absorption of vitamin B₁₂, and luminal bacteria are more abundant in the distal ileum than in the proximal small intestine.

Severe deficiency of vitamin B₁₂ produces morphologic changes in a number of sites other than in the bone marrow. Reversible nuclear enlargement of buccal⁷ and gastric⁸ epithelial cells has been described in untreated pernicious anemia. With the advent of small intestinal biopsy techniques it has been possible to examine the effect of vitamin B₁₂ deficiency on the jejunal mucosa. In a careful quantitative study of the intestinal mucosa of eight patients with untreated pernicious anemia, P. Faroozan and J.S. Trier described reduced mitoses in the germinal crypts, shortened villi, and nuclear enlargement of surface epithelial cells as well as increased cellularity of the lamina propria.⁹ Following treatment with vitamin B₁₂, mitoses rapidly increased to a normal number,

whereas several months were required before normal villus height was observed. The authors proposed that cells undergoing mitosis have the greatest requirement for vitamin B₁₂ as a co-factor of DNA synthesis, while shortened villus height is an effect secondary to partial arrest of epithelial cell germination in the crypts. Similar observations were made by A.S. Pena et al. who described a spectrum of change between patients in remission and those with untreated pernicious anemia.¹⁰ In addition to villus shortening and megalocytosis of surface epithelial cells, the authors found a general depression of disaccharidase activity in the mucosal specimens of patients with untreated pernicious anemia.

In contrast to the intestinal changes found in patients with vitamin B₁₂ deficiency, the effects of folate deficiency on the small intestine are less clearly defined. Study of folate deficiency is complicated by the frequent accompaniment of the multisystemic effects of alcoholism. Nutritional folate deficiency is often transient and rapidly reversible by folates available in the average hospital diet. Since folate is primarily absorbed in the proximal small intestine, the jejunal mucosa may have a preferential advantage for utilization of dietary folate. Several clinical observations suggest that the function of the small intestine may be partially regulated by folates.

Tropical sprue is a disease of the small intestinal mucosa characterized by partial villus atrophy and generalized intestinal malabsorption with megaloblastic anemia. In quantitative studies of the mucosal lesion in small intestine biopsies from patients with tropical sprue, V.L. Swanson and R.W. Thomassen described decreased mitoses with enlargement of crypt cell nuclei,¹¹ similar to the abnormalities described by Faroozan and Trier in pernicious anemia.⁹ Subsequently, Swanson et al. showed that treatment with either oral folic acid or parenteral vitamin B₁₂ was followed within days by increased mitotic activity and return to normal size of

epithelial cell nuclei.¹² These observations imply that the mucosa in tropical sprue may be in fact deficient in folate and/or vitamin B₁₂. However, when folic acid is administered as the sole form of treatment in tropical sprue, the hematologic lesion is usually corrected while there is only a partial improvement of intestinal absorption.¹³ Thus it is likely that the intestinal lesion in tropical sprue reflects an altered metabolism rather than simple deficiency of vitamin B₁₂ and folic acid.

Abnormal absorption of vitamin B₁₂ measured by the Schilling test was reported in two patients with nutritional folate deficiency, one an alcoholic male and the other a pregnant female with megaloblastic anemia.¹⁴ Others suggested in isolated case reports that nutritional folate deficiency may be associated with reversible intestinal malabsorption, though in most instances the cases were complicated by alcoholism, contraceptive use, or previous residence in the tropics which suggested delayed tropical sprue.^{15,16} On the other hand, S.J. Winawer et al. found normal intestinal absorption (fecal fat excretion and D-xylose absorption) and normal histology of the small intestinal mucosa which included measurements of crypt cell nuclear size in six patients with severe nutritional folate deficiency and megaloblastic anemia.¹⁷

When intestinal abnormalities have been described folate deficiency has usually been accompanied by severe chronic alcoholism. A. Bianchi et al. described enlarged crypt cell nuclei in an alcoholic patient with severe folate deficiency and megaloblastic anemia.¹⁸ These observations were expanded by J.A. Hermos et al. who compared histologic changes in intestinal biopsies obtained from three severely folate deficient alcoholics with megaloblastic anemia with biopsies from alcoholic patients with less severe folate deficiency.¹⁹ In the severely deficient patients, the biopsies showed villus shortening, decreased mitoses in the crypts, and enlargement of epithelial cell nuclei—changes essentially similar to those described in pernicious anemia.⁹ Treatment

of folate deficiency corrected the intestinal abnormalities. In six of the eight patients with less severe folate deficiency, the intestinal mucosa was essentially normal. Megalocytic changes in the mucosal epithelium with decreased mitoses in the crypts have also been induced by dietary folate deficiency in rats.²⁰

C.H. Halsted et al. studied the relationship of alcoholism, folate deficiency, and intestinal malabsorption in a prospective manner in four alcoholic volunteers.²¹ A folate deficient diet was administered to three patients, two of whom drank alcohol during the experiment and one who did not. A fourth patient drank alcohol in excess for three weeks with the hospital diet. Intestinal absorption was measured at intervals using the triple lumen jejunal perfusion method. In two patients in whom the diet was accompanied by excessive alcohol ingestion, development of a megaloblastic bone marrow was accompanied by decreased jejunal uptake of fluid, glucose, and radioactive folic acid as well as decreased absorption of D-xylose, while the intestinal mucosa remained normal. No change in absorption was found in the sober patient in whom a megaloblastic bone marrow was induced by a folate deficient diet or in the other patient who drank excessive amounts of alcohol with a hospital diet. The findings suggested that folate deficiency in combination with alcohol ingestion induces a functional abnormality of the small intestine. Since no morphologic changes were observed, the findings suggest that functional abnormality precedes structural alteration in alcoholic folate deficiency.

In summary, with vitamin B₁₂ deficiency or severe folate deficiency, megalocytic changes may be found in the small intestinal epithelium, in association with decreased mitoses in the crypts and shortened villi. In the vitamin B₁₂ deficiency of pernicious anemia, increased intraluminal bacteria are found in the upper small intestine as a consequence of achlorhydria. It is unlikely that these bacteria play a role in the intestinal mal-

absorption of the B₁₂-intrinsic factor complex. Nutritional folate deficiency appears to have a variable effect on intestinal mucosal structure and function. The effect may be modified significantly by other host factors such as concomitant alcohol ingestion. More studies are required to determine the frequency and extent of intestinal abnormality in severe nutritional folate deficiency. The nutritional consequences of the effect of B₁₂ and folate deficiency on the gut await further definition. □

1. F. Haurani, W. Sherwood, and F. Goldstein, *Metabolism* 13: 1342-1348, 1964
2. V. Herbert in *Hematopoietic and Gastrointestinal Investigations with Radionuclides*. A.J. Gilson, W.M. Smoak, and M.B. Weinstein, Editors, pp. 287-293, Thomas, Springfield, Illinois, 1972
3. J.J. Corcino, S. Waxman, and V. Herbert, *Am. J. Med.* 48: 562-569, 1970
4. R. Carmel and V. Herbert, *Ann. Int. Med.* 67: 1201-1207, 1967
5. J. Lindenbaum, J.F. Pezzimenti, and N. Shea, *Ann. Int. Med.* 80: 326-331, 1974
6. W. Sherwood, F. Goldstein, F. Haurani, and C.W. Wirtz, *Am. J. Dig. Dis.* 9: 416-425, 1964
7. M.M. Boddington and A.I. Spriggs, *J. Clin. Path.* 12: 228-234, 1959
8. B. Mäsey and C. Rubin, *Am. J. Med. Sci.* 227: 481-492, 1954
9. P. Faroozan and J.S. Trier, *New Engl. J. Med.* 277: 553-559, 1967
10. A.S. Pena, S.T. Callender, S.C. Truelove, and R. Whitehead, *Brit. J. Haematol.* 23: 313-321, 1972
11. V.L. Swanson and R.W. Thomassen, *Am. J. Path.* 46: 511-551, 1965
12. V.L. Swanson, M.S. Wheby, and T.M. Bayless, *Am. J. Path.* 49: 167-191, 1966
13. F.A. Klipstein in *Tropical Sprue and Megaloblastic Anemia*. Pp. 129-158, Churchill, London, 1971
14. R.B. Scott, R.B. Kammer, W.F. Burger, and F.G. Middleton, *Ann. Int. Med.* 69: 111-114, 1968
15. D.W. Dawson, *J. Clin. Path.* 24: 131-135, 1971

16. J. Forshaw, *J. Clin. Path.* 22: 551-553, 1969
 17. S.J. Winawer, L.W. Sullivan, V. Herbert, and N. Zamcheck, *New Engl. J. Med.* 272: 892-895, 1965
 18. A. Bianchi, D.W. Chipman, A. Dreskin, and N. Rosensweig, *New Engl. J. Med.* 282: 859-861, 1970
 19. J.A. Hermos, W.H. Adams, Y.K. Liu, L.W. Sullivan, and J.S. Trier. *Ann. Int. Med.* 76: 957-965, 1972
 20. F.A. Klipstein, S.D. Lifton, and E.A. Schenk, *Am. J. Clin. Nutrition* 26: 728-737, 1973
 21. C.H. Halsted, E.A. Robles, and E. Mezey, *Gastroenterology* 64: 526-532, 1973
-

Nutrition Reviews

authoritative articles by distinguished international scientists • competent, critical, and knowledgeable reviews • interpretative summaries of important original clinical and experimental research • selected excerpts from classic publications marking milestones in nutrition research • informative special reports on government and organization actions • contributing editors review journals of widespread interest covering biochemistry, food science, public health, toxicology, dentistry, pediatrics, internal medicine, gastroenterology and other clinical specialties •

Send subscription and renewal requests to:

THE NUTRITION FOUNDATION
489 Fifth Avenue
New York, New York 10017

\$12.00 per year

\$6.00 for students

OBESITY, JEJUNO-ILEAL BYPASS AND DEATH

Jejuno-ileal shunts for obesity can result in death years later due to failure of the liver.

Key Words: liver, cirrhosis, anastomosis, diarrhea, malnutrition

The intestinal bypass operation for treatment of obesity has passed through several stages from the anastomosis of the jejunum to the colon (associated with many complications including liver disease) to modifications resulting in the jejuno-ileal anastomosis with bypass of certain lengths of small intestine. It is now considered by some to be a fairly safe operation. Cirrhosis and death, however, have been reported after jejuno-ileal shunt for obesity.¹⁻³

A recent report concerned a white woman, 41 years old, 59 inches tall, who had been obese since adolescence. She had a history of heavy drinking but had no documented hepatic complications from alcohol. She weighed 260 pounds when she underwent jejuno-ileal bypass. An end to side anastomosis of 17 cm of jejunum to the terminal 6 cm of ileum was done. The operation bypassed 397 cm of small bowel which was left in the abdomen as a blind loop. Before the operation her lab values for albumin, globulin, bilirubin, SGOT, LDH, and BSP retention were normal. Alkaline phosphatase was mildly elevated. The liver appeared normal at surgery; it was not biopsied. After discharge, she resumed her previous high calorie, high carbohydrate, low protein diet. Five months later, when she was admitted to the hospital for diarrhea, small bowel malabsorption was not present; the diarrhea subsided during hospitalization.

Three months later she was admitted again with diarrhea, weighing only 120

pounds. She demonstrated pellagra-like skin lesions, smooth magenta tongue, brittle nails, palmar erythema, spider nevi, hepatomegaly, and edema. Albumin levels were 2.3 gm per 100 ml, prothrombin time elevated, bilirubin 2.4 mg per 100 ml, SGOT 203, LDH 425, and alkaline phosphatase 203 IU (normal level 85 IU). A liver biopsy demonstrated severe fatty metamorphosis with minimal portal scarring but no hepatocellular necrosis or regenerative nodule formation was noted. Her diarrhea subsided, she gained weight, and liver function improved on the hospital diet. She was considered for reanastomosis of the blind loop but it was not done.

Four months later she was admitted with severe protein malnutrition and hypovitaminosis. She was weak, demonstrating gross muscle wasting, cheilosis, tender feet, depigmentation of hair, gross ascites, peripheral edema, and hepatomegaly. Albumin was only 1.5 g per 100 ml, subsequently decreasing to a level of 0.6 g per 100 ml. Prothrombin time remained elevated; it was not corrected by vitamin K. Bilirubin was 4 mg per 100 ml, SGOT 101, alkaline phosphatase 131 IU, potassium concentration had decreased to 2.7 mEq per liter, and blood urea nitrogen was at a very low level of 2 mg per 100 ml. Phosphorus, calcium, and magnesium were all decreased in concentration. At the time of admission, she would eat only candy. Nasogastric tube feedings produced diarrhea and volume ranged as high as 4 liters per day. Intravenous total parenteral nutrition produced hepatic coma with hyperammonemia on three separate occa-

sions, despite slow administration of the solution and bowel sterilization. Discontinuation of amino acids in the infusate produced recovery, but she was still considered a poor operative risk for the anastomosis. A fourth episode of hepatic coma associated with gastrointestinal bleeding occurred despite oral antibiotic therapy and low protein intake. She died two months after hospital admission and the premortem laboratory values were: bilirubin, 8.8 mg per 100 ml; alkaline phosphatase, 113; LDH, 197; prothrombin time elevated; albumin 0.6 per 100 ml (despite intravenous albumin given in large quantities). Blood cultures one week before death were positive for *Candida albicans*.

Autopsy findings revealed an emaciated woman with marked muscle wasting. She was jaundiced with ascites (2500 ml), splenomegaly, esophageal varices, and a healing gastric ulcer. The liver was enlarged, weighing 1450 g. On cut section, the liver demonstrated a green surface interspersed with light yellow fibrous bands running in all directions. There was no evidence of bile duct obstruction. There was generalized moniliasis, and cerebral atrophy. On microscopic examination sections of the liver revealed cellular necrosis, parenchymal cell loss, cholestasis, and bile duct proliferation. Lymphocytic infiltration in portal areas with moderate fatty change was noted. A marked fibrosis of a diffuse nature with bands linking portal triads was present, and regenerative nodule formation was noted.

The jejunio-ileal shunt operation, therefore, is associated with pathologic changes which can produce death some time after the operation.³ Twenty-five months after the bypass operation the liver of the subject of this report passed through stages of fatty metamorphosis to cirrhosis and death. Although it was thought that the individual was drinking through the first two of the 25 months, it was felt that alcohol was not solely responsible for hepatic changes. She definitely was not taking alcohol during her last three months while she was hospitalized, during which time liver failure developed rapidly. She had consumed a

poor diet which had produced malnutrition and diarrhea during the period after the operation.

There are now seven deaths reported from liver disease in 63 patients with jejunio-colic anastomosis. Benign steatosis has been shown in a number of reports after jejunio-ileal shunts for obesity but this present case marks only the second case in which definite cirrhosis occurred after the operation, which was linked with death.

It appears that the changes in liver which have been reported are a function, in some regard, of the length and type of small bowel bypass and anastomotic site. The 200 cm ileal bypass for the treatment of hypercholesterolemia is not associated with a significant loss of weight or known complications.

Several possibilities for the hepatic injury after jejunio-ileal bypass are hepatic toxicity of absorbed secondary bile salts such as lithocholic acid, the absence of lipotropic factor which is absorbed due to the malnutrition associated with the operation, protein malnutrition, and the blind loop syndrome.

Thus there is no doubt that severe liver damage can result from the jejunio-ileal bypass for obesity. Although liver problems in this case were compounded by poor choices of food by the subject, the question arises as to how many deaths of this type have occurred. Many of the people who have had jejunio-ileal bypass operations have had them taken down and thus, the natural history of liver changes with this disorder cannot be accurately documented. It may be that most obese people have mild to moderate hepatic steatosis prior to operation and that these changes are accentuated and can eventually result in cirrhosis as a result of the surgical procedure. In some people with jejunio-colic shunts, fat accumulation in the liver was associated with inflammation, leading to cirrhosis and liver failure and occasionally death.³ These changes may be reversed if anastomosis is taken down and normal intestinal continuity restored.

The case reports and the documented problems associated with liver failure indicate that if there is a place for the jejuno-ileal shunt in the treatment of obesity, it should be reserved for massive obesity which cannot be resolved in any other way. Perhaps these individuals should be followed repeatedly with liver biopsies and the shunt taken down when evidence of histological changes suggestive of impending cirrhosis occur.

More data are needed on the natural history of people who survive the operation. For instance, do they begin to gain weight once again if they do not develop liver failure? If the liver is protected by the length of shunt involved operatively, then it may well be that these people will eventually begin to gain weight again if the segment of bypassed gut is decreased. Unfortunately, this type of surgical procedure can be performed by any qualified surgeon and most likely, the procedure is being done more frequently than reported. The possibility of severe changes produced in the liver after a fairly long period of time leading to death, makes this a procedure which must be regulated in some fashion by physicians caring for patients with obesity. A cure for obesity has been estimated to advance life span five years. We do not know the five year or lifetime sur-

vival rates of people with this operation. Many of the individuals who are operated on for the jejuno-ileal shunt must also consider a lifetime of living with chronic diarrhea — it may not be worth it.

This case history also reveals the improvement of hepatic coma obtained with a low protein diet. One question arises, not related to this particular case, but to the general problem of treatment of cirrhosis: Would low protein diets as are currently prescribed for patients with chronic renal disease be helpful? Compensated cirrhotics usually are discharged from hospitals with no diet prescription at all and with a projected life span of one to ten years. Since gastroenterologists have not added nutrition to their sub-specialty, the treatment of cirrhotics with controlled protein diets should be seriously considered by them. □

-
1. Current Status of Jejuno-Ileal Bypass for Obesity. *Nutrition Reviews* 32: 333-336, 1974
 2. J.C. Mangla, W. Hoy, Y. Kim, and M. Chopek: Cirrhosis and Death after Jejuno-Ileal Shunt for Obesity. *Am. J. Dig. Dis.* 19: 759-765, 1974
 3. D. B. McGill, S. R. Humphreys, A. H. Baggenstoss, and E. R. Dickson: Cirrhosis and Death after Jejuno-Ileal Shunt. *Gastroenterology* 63: 872-877, 1972

MASSIVE OBESITY AND NEPHROSIS

Massive obesity can produce the nephrotic syndrome which disappears after the loss of body weight.

Key Words: proteinuria, nephrotic syndrome, weight loss, lung volume, edema

Obesity is associated, perhaps causally, with many disorders; hypertension, joint disease, diabetes mellitus, right heart failure, and hyperlipidemia, for example. It decreases respiratory function and increases surgical risk. A recent report¹ appears to demonstrate that massive obesity can produce the nephrotic syndrome.

Four cases were studied. The first was a 25-year-old woman who had a negative history for chronic renal disease. There was no familial history of obesity or renal disease and she became obese after contracting poliomyelitis. The only complication of massive obesity on initial examination was bilateral pitting edema of her feet and legs. Several random urine samples showed a 4+ proteinuria.

The second case was a 49-year-old man, eating approximately 6000 calories daily. Family history was negative for heart or kidney disease. He weighed 179 kg and was plethoric. His blood pressure was 180/110 mm Hg and 2+ pitting edema of his legs was present. Proteinuria was present.

The third subject, obese since childhood, also showed edema of the legs. The patient fell asleep during the examination. Arterial hypoxemia and hypercapnea were exacerbated by sleep which was accompanied by periodic breathing and apnea.

The fourth patient was a 49-year-old woman with hypertension and 2+ pitting edema with proteinuria.

The studies in the research center were formulated to define the relationship between diet, weight loss, and proteinuria. A formula diet was fed which provided 500 kcal containing 23 g of fat, 54 g of carbohydrate, 19 g of protein, and 20 mEq of sodium. No medication was permitted except for an occasional sedative.

Before the weight loss there was heavy proteinuria in all subjects ranging from 3 to 19 g per 24 hours. Most of the protein was albumin (73 to 96 percent). The serum albumin concentrations were low, ranging from 1.7 to 3.1 g per 100 ml. The serum cholesterol concentrations were elevated. All patients had pitting edema and there was no significant alteration in proteinuria related to posture or exertion in three of the subjects. The creatinine clearances were normal. The glomerular filtration rate and effective renal plasma flow were also normal despite the heavy proteinuria. A battery of tests ruled out such possible causes of nephrosis as lupus erythematosus, diabetes mellitus, and immune reactions.

A high correlation was demonstrated between the measured body weight and the quantitative proteinuria. As the body weight decreased, the proteinuria decreased and the correlation coefficients for the four patients varied between 0.81 and 0.95.

There was no effect of dietary depletion of protein on urinary excretion of protein, nor was there any relationship between restricted sodium and the decrease in

proteinuria. No change in the proteinuria occurred when the dietary protein was increased (total kcal remaining at 500) or when the sodium was increased in the diet.

Tests of the cardiac function revealed no signs of congestive failure. The blood pressures were normal in the hospital but mild dyspnea on exertion was present. No orthopnea or nocturnal dyspnea was found. The cardiac output, however, was high, ranging between 7 and 20 liters per minute. Oxygen consumption was elevated, although most likely appropriate for their body weight. A normal arteriovenous oxygen difference was present but the right atrial pressure was elevated, ranging from 10 to 20 mm Hg. In two subjects right atrial and/or superior vena cava pressure returned to normal with a weight loss, and associated with this was a decrease in proteinuria to less than 1 g per 24 hours.

The lung volumes were decreased in three subjects, consistent with the values reported for other people with massive obesity. Two of the three patients extensively studied showed an elevated arterial carbon dioxide pressure along with decreased partial pressure of oxygen levels. After the weight loss, the lung volumes increased and the arterial blood gas levels improved markedly.

The hematocrit, hemoglobin, erythrocyte mass, and plasma volume were elevated during massive obesity. After the weight loss, the hematocrit, hemoglobin, and blood volume decreased.

Glomerular abnormalities and focal tubular atrophy were noted in the renal biopsy specimens. Some of the tubules contained hyaline droplets. Immunofluorescent staining was positive in the mesangium. Electron microscopy also demonstrated pathologic changes in the renal architecture.

Plasma glucose levels were only mildly elevated. Diabetic glomerular sclerosis appeared unlikely because of the mild hyperglycemia, and the anatomic structure showed no evidence of these changes. Since remission of nephrosis was positively related to weight loss, proteinuria with

glomerular and tubular lesions in massive obesity represents a renal response to a circulatory disorder which, most likely, is reversible. Repeat biopsies, however, were not done to substantiate this impression.

The study points out well the seriousness of massive obesity with increased right heart blood pressure, decreased lung volume, increased circulating blood volume, abnormal blood gases, edema, and renal pathology. Despite the seriousness of this condition, however, three of the four patients regained body weight when dismissed from the hospital and the nephrotic syndrome appeared again.

One of the most difficult syndromes to treat in human medicine is obesity, despite

the fact that the cause and treatment are supposedly known. Results are generally discouraging. In these four cases, in which both obesity and kidney disease were present, the response to dietary therapy was poor. This response tends to confirm the clinical impressions that in the rare cases of obesity and kidney disease, dietary treatment meets with much less success than when treatment is only for renal disease. □

-
1. J. R. Weisinger, R. L. Kempson, F. L. Eldridge, and R. S. Swenson: The Nephrotic Syndrome: A Complication of Massive Obesity. *Ann. Int. Med.* 81: 440-447, 1974

LACTATION AND COMPOSITION OF MILK IN UNDERNOURISHED WOMEN

The chemical composition of breast milk of undernourished mothers is not significantly altered and human milk is the sole food for infants in many traditional societies. Rapid urbanization carries with it the risk of inadequate breast feeding and development of infantile marasmus in these communities.

Key Words: breast milk, breast feeding, under-nutrition

Despite the socio-cultural pressures of a rapidly spreading industrial society, a vast majority of infants in different parts of the world are reared during the first year of their life, either solely or mainly, on mother's milk.¹ This usually happens in economically backward areas where malnutrition is rampant. The growth pattern of these infants during the first six months of life is fairly good,² indicating a satisfactory yield of breast milk. This is surprising, considering that the mothers themselves subsist on inadequate diets and suffer from various nutritional deficiencies. It has hence been suggested that the milk-synthesizing machinery adapts well to ill-balanced diets.^{3,4}

The study of the composition of milk in undernourished mothers has therefore been a topic of considerable interest to nutritionists. The latest in these series is the report by B. S. Lindblad and R. Rahimtoola.⁵ Ten young Pakistani mothers belonging to the lower socio-economic group and lactating for 1.5 to 6 months were studied. They were mothers of infants admitted to the hospital for diarrhea or respiratory infections. Milk was collected by pumping the breasts and analyzed for protein, fat, and lactose.

The concentration of protein and lactose were found to be normal in these milk samples. This observation is in line with earlier reports from South India⁶ and Papua, New Guinea.⁷ The concentration of fat and vitamin A were also found to be normal. A low concentration of these

nutrients was reported from South India^{6,8} whereas a normal fat content was observed in milk from Egyptian mothers.⁹ P. S. Venkatachalam⁷ had suggested that the fat content of breast milk might be related to the fat intake of the mother. Recent studies indicate that the total calorie intake is probably the most limiting factor in the diets of economically backward regions.¹⁰ The extent to which calorie intakes influence the composition of milk remains a relatively unexplored field, partly because of the preoccupation of nutritionists with protein supplementation.^{6,8}

Earlier data indicating alterations in protein quality have also been published.^{8,11,12} The present authors report decreased levels of lysine and methionine, an observation contrary to those made previously.

An important observation to which Lindblad and Rahimtoola made only a fleeting reference is the total milk output. Although no figures have been presented, they state that the milk volume was low. As has been indicated, however, surprisingly good yields of breast milk have been reported in undernourished mothers.^{3,4,7,13}

The importance of breast feeding particularly in economically backward communities cannot be overemphasized. B. S. Platt¹⁵ was one of the first to point to its significance in the reduction of protein-calorie malnutrition. □

1. D. B. Jelliffe in *Infant Nutrition in the Subtropics and Tropics*. 2nd Edition, pp. 32-84. World Health Organization, Geneva, 1968
2. C. Gopalan: Protein Intake of Breast-Fed Poor Indian Infants. *J. Trop. Pediat.* 2: 89-92, 1956
3. A. R. P. Walker, U. B. Arvidsson, and W. L. Draper: Breast-Feeding and Diet. *Lancet* 1: 317, 1952
4. M. Gunther: Breast-Feeding and Diet. *Lancet* 1: 367, 1952

5. B. S. Lindblad and R. Rahimtoola: A Pilot Study of the Quality of Human Milk in a Lower Socio-Economic Group in Karachi, Pakistan. *Acta Paediatr. Scandinav.* 63: 125-128, 1974
6. C. Gopalan and B. Belavady: Nutrition and Lactation. *Fed. Proc.* 20 (Supplement 7): 177-184, 1961
7. P. S. Venkatachalam in *A Study of the Diet, Nutrition and Health of the People of the Chimbut Area*. Monograph 4, Dept. Public Health, Govt. of Papua, New Guinea, 1962
8. A. K. Deb and H. R. Cama: Studies on Human Lactation. Dietary Nitrogen Utilization during Lactation and Distribution of Nitrogen in Mother's Milk. *Brit. J. Nutrition.* 16: 65-73, 1962
9. M. M. Hanafy, M. R. A. Morsey, Y. Seddick, Y. A. Habib, and M. elLozy: Maternal Nutrition and Lactation Performance. *J. Trop. Pediat. and Environ. Child Health* 18: 187-191, 1972
10. P. V. Sukhatme and D. Basu: The Present Pattern of Production and Availability of Foods in Asia in Proceedings of the First Asian Congress of Nutrition, pp. 19-45, Nutrition Soc. India, Hyderabad, 1972
11. P. R. Srinivasan and M. K. Ramanathan: Protein and Sulphur Amino Acids in Breast-Milk of Poor Class Indian Women in the Nilgiris. *Indian J. Med. Res.* 42: 51-54, 1954
12. M. G. Karmarkar, J. Kapur, A. D. Deodhar, and C. V. Ramakrishnan. Studies on Human Lactation I. Diet Survey of Lactating Women in Different Socio-Economic Groups and the Effects of Socio-Economic Status and Stage of Lactation on the Proximate Principles and Essential Amino Acids of Human Milk. *Indian J. Med. Res.* 47: 344-351, 1959
13. C. Gopalan: Studies on Lactation in Poor Communities. *J. Trop. Pediat.* 4: 87-97, 1958
14. D. B. Jelliffe and E. F. P. Jelliffe: II. Special Problems in Developing Countries. The Urban Avalanche and Child Nutrition. *J. Am. Dietet. Assn.* 57: 114-118, 1970
15. B. S. Platt: The Malnourished Community. Care of Mothers and Children as a First Step Towards Improved Feeding. *Lancet* 1: 929-930, 1954

SUCROSE, STARCH AND HYPERLIPIDEMIA

In two- to three-week studies with young men an excessive consumption of energy was associated with hyperlipidemia when sucrose replaced the starch in the diet

Key Words: sucrose, starch, blood lipids, energy intake, cholesterol, triglycerides

Large loads of dietary carbohydrate have been shown by several workers¹ to have effects on the blood lipid pattern. Many of these studies involved the comparison of the effects following starch or sucrose ingestion. These studies show that sucrose loads induced a rise in the plasma lipids. Other studies on plasma lipids compared the effects of complex (i.e. polysaccharides) carbohydrates in the diet with diets containing simple sugars either as mono- or disaccharides.² Some studies suggested that part of the difference between a polysaccharide such as starch and simple sugars may be due to differences in the rate of digestion and absorption although a strong lipogenic effect of the fructose moiety of sucrose was also present.³

The results obtained in other studies have not been so clear cut and the position has been clouded by the development of controversy regarding the significance of these findings in the etiology of coronary heart disease. Some work suggested that the hyperlipidemia observed when excessive sucrose is consumed is due to an increased energy intake and not to the source per se.⁴ In a recent paper D. J. Naismith and co-workers⁵ describe the results of three experiments in which the interrelations of energy intake and sucrose versus starch effects have been studied.

The subjects were healthy male students who were subjected to close dietary supervision during the course of the study. In the first study 23 subjects consumed their self-selected diet for seven days; a propor-

tion of the starch in their customary diet was then replaced with an equicaloric amount of sucrose. This was done so that other changes in the diet were kept to a minimum. The exchange involved approximately 200 g of starch. The subjects consumed these modified diets for a further 14 days after which they returned to their usual diet for another 14 days. Blood was taken at the beginning and end of the first seven days, and at the ends of the two other dietary treatments and analyzed for total cholesterol, triglycerides, and phospholipids. A second identical study with ten subjects was then carried out in which the blood cholesterol was fractionated into free and esterified components.

In the third experiment involving 12 subjects, the energy intake of each subject was increased by 1800 kcal per day for 21 days using the usual components of their diet. Six of the subjects had their sucrose intake reduced during this overfeeding and the remainder had it raised by dietary manipulations.

In the first experiment the energy intakes on the high sucrose diet were, on the average, virtually the same as on the self-selected diet. The sucrose intake was trebled, increasing from 100 to 300 g per day, and the proportion of the dietary energy from carbohydrate increased slightly at the expense of the energy from fat. The blood lipids showed significant rises in total cholesterol, triglycerides, and phospholipids on the high-sucrose diet and the concentrations returned to the preliminary levels on return to the subjects' usual diets.

The results from the second study were very similar. Fractionation of cholesterol showed that the rise occurred almost exclusively in the esterified fraction.

In the third experiment the six subjects on the low-sucrose diet increased their energy intake by some 1700 kcal per day, their starch intake increased from 123 to 221 per day, and the sucrose intake fell from 73 to 41 g. The proportion of energy from carbohydrate on the diet fell and was accompanied by an increased fat intake. In the other six subjects energy intake was increased by a similar amount, starch intake was reduced from 172 to 45 g, and sucrose increased from 110 to 329 g. The changes in the proportions of energy from carbohydrates and fat were similar to those in the low-sucrose group.

The triglyceride levels rose significantly during the over-eating on the high-sucrose diet and returned to normal levels when the subjects returned to their normal diet. The changes on the low-sucrose diet were not significant although a small rise did occur. The changes in the plasma cholesterol values were similar, being about 50 percent above the normal levels during over-eating the high-sucrose diet and again returning to normal on the normal diet. No changes of significance occurred on the low-sucrose diet.

The results obtained in this study demonstrate a hyperlipidemic effect associated with the replacement of starch in the diet with sucrose. These changes occurred in the three major lipid fractions in the blood and fractionation of the total cholesterol showed that the rise was confined to the esterified fraction.

Increasing the energy intake by 60 percent above normal had little or no effect on the plasma triglycerides or cholesterol unless it was associated with an increased sucrose intake. The group receiving the low sucrose diet had an increased fat intake of some 120 g per day, yet this did not produce any hyperlipidemic effect.

This paper shows that increased consumption of sucrose compared with starch has a significant effect on the plasma lipids

and that this effect is not related to the level of energy intake.

The practical significance of these kinds of studies remains obscure. Although sucrose and starch are normal constituents of practically all diets, the consumption of as much as 300 g of sucrose must be relatively rare. The question remains whether variations in the amount of sucrose consumed by most individuals has any significant effect upon blood lipid levels. The permanence of carbohydrate induced changes in blood lipid levels is also a matter of debate.⁶ A better understanding of metabolic pathways responsible for the effects of different carbohydrates upon serum lipids would be helpful in resolving the questions repeatedly raised in this area. □

1. I. MacDonald and D. M. Braithwaite: The Influence of Dietary Carbohydrate on the Lipid Pattern in Serum and Adipose Tissue. *Clin. Sci.* 27: 23-30, 1964
2. M. A. Antar and M. A. Ohlson: Effect of Simple and Complex Carbohydrates upon Total Lipids, Nonphospholipids, and Different Fractions of Phospholipids of Serum in Young Men and Women. *J. Nutrition* 85: 329-337, 1965
3. D. J. Naismith and I. A. Rana: Sucrose and Hyperlipidaemia. II. The Relationship between the Rates of Digestion and Absorption of Different Carbohydrates and Their Effect on Enzymes of Tissue Lipogenesis. *Nutrition Metab.* 16: 285-294, 1974
4. J. I. Mann and A. S. Truswell: Effects of Isocaloric Exchange of Dietary Sucrose and Starch on Fasting Serum Lipids, Postprandial Insulin Secretion and Alimentary Lipaemia in Human Subjects. *Brit. J. Nutrition* 27: 395-405, 1972
5. D. J. Naismith, A. L. Stock, and J. Yudkin: Effects of Changes in the Proportions of Dietary Carbohydrates and in Energy Intakes on the Plasma Lipid Concentrations in Healthy Young Men. *Nutrition Metab.* 16: 295-304, 1974
6. R. B. McGandy, D. M. Hegsted, and F. J. Stare: Dietary Fats, Carbohydrates and Atherosclerotic Vascular Disease. *New Engl. J. Med.* 277: 417-419, 469-471, 1968

PROBLEMS IN IRON ENRICHMENT AND FORTIFICATION OF FOODS

The enrichment and fortification of foods with iron faces a number of biological and technological problems. The bioavailability of iron compounds is not easily predictable in the food product and the most available forms of iron cause the greatest difficulty in quality and storage of the target food.

Key Words: iron sources, bioavailability, fortification

There is general agreement on the need for increasing the level of iron enrichment of flour, cereal products, breads, buns, and rolls.^{1,2} An additional approach to the problem of iron deficiency anemia might include fortification of an even wider variety of foods, including non-cereal products. Further support for additional vehicles for iron enrichment comes in the recent increase of the RDA for iron for pregnant women.³

Among considerations of the Food and Nutrition Board and the Council on Foods and Nutrition which led to support of an iron enrichment program was "the technologic feasibility of improving the dietary by enrichment".¹ In actual fact this feasibility is not as straightforward as it seems. Recent publications on the bioavailability of iron sources indicate that availability is not to be taken for granted and that the interactions with components of food during processing is complex, and presently unpredictable.^{4,5} In addition, there is reason to believe that the most available forms of iron are the most problematic from the point of view of compatibility with the organoleptic qualities and the stability of other nutrients in the product.^{6,7}

The iron compounds approved for use as food additive sources of dietary iron are: ferric orthophosphate, ferric pyrophosphate, ferric sodium pyrophosphate,

ferrous gluconate, ferrous lactate, ferrous sulfate, ferrous fumarate, and reduced iron. Unfortunately, approval of this type does not necessarily guarantee that the added compound will be efficacious. In fact a great deal of recent evidence would tend to indicate that the various ferric phosphates are poorly or not at all available.^{4,5} The more commonly used ferrous salts and reduced iron fare slightly better as additives, but in general the availability was less than that of the natural iron in the products.⁴ The authors conclude "that the availability of the iron in the fortified product depended either upon the nature of the product or perhaps on manufacturing procedures" and that "the data clearly demonstrate the necessity of assessing iron availability in final products and that the utility of iron added cannot be determined by prior test of the material which is added". In this latter study, products tested included cocoa powder, infant formula, bread, and breakfast cereals.

Some of the technical problems of iron fortification are due to the many chemical reactions which are iron catalyzed or in which iron is consumed as a reactant. Among these reactions are those which involve a variety of sulfur compounds, leading to black insoluble deposits or a gray appearance of the product. This type of reaction might in fact contribute to the loss of availability found in processing. Iron is also known to accelerate the oxidation of ascorbic acid and to catalyze the autoxidation

tion of unsaturated lipids.⁸ In the latter case this will also lead to off-flavors and the loss of beta-carotene and tocopherols.

Some of these problems are exemplified in a recent attempt to fortify dehydrated mashed potatoes.⁶ This product was chosen because of its importance as a source of calories and its inclusion in U.S. Department of Agriculture assistance programs. A number of iron sources were included in the study, ranging from ferrous and ferric salts to polyphosphate gels and a ferriphosphate-whey protein complex. Color problems were significant even at low levels of enrichment (2 to 5 mg Fe per 100 g). The addition of iron compounds to both fresh and reconstituted dehydrated mashed potatoes usually resulted in the rapid development of a dark gray-green discoloration. The intensity of color formation varied greatly for the same compound in different potato products and appeared unrelated to the process or to geographic origin. Similar difficulties were found with the flavor of the fortified product, and gas chromatographic data indicated that the fortified product contained higher levels of major volatile oxidation products than the unfortified control. Unfortunately, ascorbic acid stability was not examined nor were any "reduced iron" preparations investigated. The authors did suggest encapsulated iron as a potential feasible approach, and this would be justified on the basis of some of the results of the bioavailability study.⁴

Other technical problems have been encountered in cereal products,⁷ in addition to undesirable effects on color and flavor. For example the high density of reduced iron makes it difficult to obtain stable blends with flour, especially for modern plants which use pneumatic conveyers. The density also makes reduced iron unsuitable for the continuous bread process. The cost

of some sources of iron is also an important factor and should ultimately be considered on a "per pound of available iron" basis.

It seems obvious that more research is required on this subject, in particular coordinated efforts considering process requirements, chemical reactions in foods, and bioavailability in the product. There appear to be possibilities in encapsulated forms of iron and perhaps we need to know more about the "natural" forms of iron, which as previously mentioned, may be more available and probably more stable than many of the additives. □

-
1. W. J. Darby: The Case for the Proposed Increase in Iron Enrichment of Flour and Wheat Products. *Nutrition Reviews* 30: 98-102, 1972
 2. *Proposed Fortification Policy for Cereal-Grain Products*. Food and Nutrition Board, National Academy of Sciences - National Research Council, Washington, D. C., 1974
 3. *Recommended Dietary Allowances*. Food and Nutrition Board, National Academy of Sciences - National Research Council, Washington, D. C., 8th ed., Publ. 2216, 1974
 4. E. K. Amine and D. M. Hegsted: Biological Assessment of Available Iron in Food Products. *J. Agr. Food Chem.* 22: 470-476, 1974
 5. J. Waddell: The Bioavailability of Iron Sources and Their Utilization in Food Enrichment. *Fed. Proc.* 33: 1779-1783, 1974
 6. G. M. Sapers, O. Panasiuk, S. B. Jones, E. B. Kalan, and F. B. Talley: Iron Fortification of Dehydrated Mashed Potatoes. *J. Food Sci.* 39: 552-554, 1974
 7. Report of the Ad Hoc Committee on Iron Enrichment of Wheat Flour and Baked Foods. American Bakers Association, Washington, D.C., 1972
 8. K. U. Ingold, in *Symposium on Foods: Lipids and Their Oxidation*, H. W. Schultz, E. A. Day, and R. O. Sinnhuber, Editors, pp. 93-121. The Avi Publishing Company, Inc., Westport Connecticut, 1962

NUTRITION CLASSICS

from THE JOURNAL OF BIOLOGICAL CHEMISTRY 53:293-312, 1922

STUDIES ON EXPERIMENTAL RICKETS.

XII. AN EXPERIMENTAL DEMONSTRATION OF THE EXISTENCE OF A VITAMIN WHICH PROMOTES CALCIUM DEPOSITION.

By E. V. McCOLLUM, NINA SIMMONDS, AND J. ERNESTINE
BECKER,

(From the Department of Chemical Hygiene, School of Hygiene and Public Health, the Johns Hopkins University, Baltimore.)

AND P. G. SHIPLEY.

(From the Department of Pediatrics, the Johns Hopkins University, Baltimore.)

(Received for publication, June 20, 1922.)

With the discovery in 1913 that certain fats contain a substance or substances which are essential for growth, this class of foodstuffs assumed an importance in nutrition which had not been hitherto accorded it. At first the evidence of the existence of the substance which subsequently became known as fat-soluble A, or vitamin A, rested upon the failure of experimental animals to grow when the fats carrying this substance were lacking in the food, and the resumption of growth when such fats were administered. Later xerophthalmia of a certain type was recognized as a pathological condition which invariably results from specific starvation for fat-soluble A. We have recently, however, convinced ourselves that a similar ophthalmia may be the result of disturbance of the balance of inorganic elements in the diet. Up to the present time no definite evidence has been brought forward to show whether one or more than one substance is contained in those fats which contain fat-soluble A which gives them their unique biological value (1). The great activity in several laboratories in the study of the cause or causes of rickets and related conditions has, during the last 3 years, brought to light the fact that there is a rôle played by fats in the etiology of this disease. This observation causes us to appreciate further the importance of the fat moiety of the food supply, and emphasizes the necessity

of providing in the diet a regular and abundant supply of such fats as promote the normal development of the skeletal tissues.

Mellanby (2) was the first to associate the group of fats which, because of their content of fat-soluble A were frequently distinguished as "growth-promoting" fats with the prevention of rickets. His work focused attention upon the problem as to whether fat-soluble A is itself a substance essential for the normal growth of bone.

Mellanby (3) was so impressed with the power of butter fat to protect puppies against the abnormalities of bone growth that he stated:

"These facts of agreement from the point of view of physiological reaction seem to me strong evidence that the substance in fats stimulating the calcification of bone is the same as Fat-soluble A, i.e. the factor which stimulates growth in rats."

. . .

Our own experience had convinced us that existing methods were incapable of differentiating beyond doubt between fat-soluble A and a special calcium-depositing substance should such exist. We therefore formulated a plan which involved a comparison of a selected list of fats in respect to three kinds of effects in nutrition. First, we tested cod liver oil, shark liver oil, butter fat, and several vegetable oils for potency in causing the cure of xerophthalmia due to lack of fat-soluble A. Secondly, we made comparative tests of the same fats to determine their value in promoting growth in young rats which were restricted to a diet so low in calcium that satisfactory growth was not possible without the provision of some substance which would make for a greater efficiency in the utilization of calcium than that which could be effected in its absence. Thirdly, we further studied these same fats by means of our "line test" to discover their relative values for inducing the deposition of the line of calcium salts in rachitic bones. With the data which we have secured from these three distinct types of tests, we are now in a position to interpret accurately the results of much of the experimental data in the literature which is otherwise confusing.

. . .

DISCUSSION OF RESULTS.

We have shown experimentally that cod liver oil oxidized for 12 to 20 hours does not cure xerophthalmia in rats. It does, however, cause the deposition of calcium in the bones of young rats which are suffering from rickets. This shows that oxidation destroys fat-soluble A without destroying another substance which plays an important rôle in bone growth.

. . .

Cod liver oil, shark liver oil, and burbot liver oil, are highly effective for curing xerophthalmia, for protecting the body against the effects of a deficiency of calcium, and for the deposition of lime salts in rachitic bones.

Certain vegetable fats, among which are cottonseed oil, maize oil, sesame oil, and olive oil, do not possess the property of curing xerophthalmia, nor do they raise the efficiency of the tissues in utilizing calcium when there is an inadequate provision, nor of initiating healing in rickets.

Butter fat contains the calcium-depositing factor but in much smaller amounts than the fish oils we have examined. It is a much better source of fat-soluble A than of the substance which regulates calcium metabolism.

. . .

The evidence set forth in this paper demonstrates that the power of certain fats to initiate the healing of rickets depends on the presence in them of a substance which is distinct from fat-soluble A. These experiments clearly demonstrate the existence of a fourth vitamin whose specific property, as far as we can tell at present, is to regulate the metabolism of the bones.

COPPER TOXICITY, RATS AND WILSON'S DISEASE

The ability to store ionic copper in tissues such as the liver and the brain may be one of the main causes of copper accumulation in these organs of patients with Wilson's disease. In rats drinking water high in copper, the organs which can take up ionic copper store it and they are not protected from copper toxicity. On the other hand, those organs which require copper bound in ceruloplasmin for storage, do not show excess copper and thus, are protected from copper toxicity.

Key Words: copper concentration, ceruloplasmin, liver, iron

The etiology of copper deposition in tissues of patients with Wilson's disease has not been clearly established. It may be due to excessive absorption of copper from the intestine, an inability to excrete copper, or inability to convert copper to ceruloplasmin. Hypotheses remain hypotheses although each has some data to support it. A search for an experimental animal which mimics Wilson's disease has led, as usual, to the rat. There is dispute, however, whether copper loading in rats is similar to Wilson's disease because consumption of great quantities of copper is not a feature of Wilson's disease. In Wilson's disease, copper levels are ten times normal or more in the liver, kidney, brain, and cornea despite normal dietary levels in food. However, only slight increases are found in spleen, lung, erythrocytes and skin, and normal or decreased concentrations are often found in other organs.¹

In long term studies of copper loading in rats extending for two and a half years, a general parallel was found between artificial copper toxicity in the rat and the tissue copper levels demonstrated in

patients with Wilson's disease. After two years, the rats' liver contained copper at levels 65 times normal, kidney and small bowel five times normal, large bowel three times normal, and brain two times normal. Copper content in lung, heart, bone marrow, testes, and erythrocytes remained almost normal. Other organs demonstrated only small increases in copper. The increase in copper in the small intestine occurred immediately after copper was placed in the drinking water and persisted for the next two years. The high concentration of copper in the small intestine of rats compared with normal or decreased levels in human beings with Wilson's disease, was most likely due to the increased copper content of drinking water given to the rats. The human gut is not exposed to high levels of dietary copper. Another difference noted was that copper concentration in hepatic bile of rats was extremely high compared with no increase in the bile of subjects with Wilson's disease. Rats were also different in that the copper concentration was increased in the skin and nails and found to be very high in the hair while in Wilson's disease these areas are usually normal with hair copper content decreased.

It was concluded that despite a normal intake of copper by patients with Wilson's disease, that the mishandling of copper by them was similar to that noted when normal rats were fed copper in excess. The data, however, did not clearly indicate which hypothesis was correct concerning the abnormality of copper metabolism in Wilson's disease.

In rats, when tracer copper in ionic form was injected intravenously, specific activity in organs was less than expected. (The expected activity was derived from data using normal rats not drinking excess copper in their water rations.) This was true even for organs that had increased copper content in the toxic rats. The highest specific activities, although still below those expected, were found in bone marrow, heart, kidney, erythrocytes, and muscle. The response was between one-half and one-third of normal. Lung, spleen, and testes were approximately one-fifth normal while brain, skin, liver, and small bowel were one-tenth of normal. Large intestine and bone were lower than one-tenth of normal.

When ceruloplasmin labeled with ^{67}Cu was injected, completely different specific activities were noted. Values were normal (again, normal judged from ceruloplasmin studies in normal rats) in the brain, heart, bone marrow, spleen, and testes and about half of normal in the kidney, lung, and muscle. Liver and other organs showed low specific activities of ceruloplasmin. The radioactive copper remained almost entirely within the ceruloplasmin molecule during the 48-hour test period. When the specific activities of ceruloplasmin were compared with ionic copper in the copper-laden rats, only the liver showed less specific activity after ceruloplasmin ^{67}Cu than after ionic ^{67}Cu . The specific activity of ceruloplasmin was greater than ionic copper in the lung, testes, skin, bone, muscle, heart, bone marrow, brain, and spleen. It was equal to ionic copper in erythrocytes and in kidney, small, and large intestine.

Because of the well-known influence of copper deficiency on mobilization of iron

stores, the iron concentrations were studied in the organs of five copper-laden rats. Tissue iron levels were significantly increased in the kidney, lung, muscle, spleen, and testes and were modestly increased in all other organs except the large and small intestine. There was no obvious parallel between changes in concentration of iron and copper.

Because injection of ionic copper failed to induce a normal specific activity, a defensive mechanism by which massive copper deposition is prevented may have been operative. If an organ of a rat accepted ionic copper, then it was that organ which accumulated a significant amount of copper during copper loading. If, however, the organ ordinarily took up ceruloplasmin preferentially, then little stable copper was accumulated.

The preferred form of copper in the brain appeared to be ionic while the liver failed to differentiate between forms of metal, ionic, or as ceruloplasmin. This failure to differentiate the form of copper or the preference for ionic copper appeared, therefore, to explain the organ accumulation of copper.

Copper toxicity induced in rats resulted in increased copper in those organs known to handle ionic copper directly; the liver, kidney, small intestine, and brain (to a lesser degree). The liver removes copper from blood to synthesize ceruloplasmin and also excretes copper into bile and stores excess. The kidney excretes copper into urine and absorption of copper occurs primarily in the upper small intestine.

The defense mechanism of some organs (identified as the requirement of ceruloplasmin formation before copper storage resulted) was imperfect because these organs did accumulate copper slowly. Organs such as lung, testes, and erythrocytes, on the other hand, which require ceruloplasmin for storage of copper, when normal levels of copper are fed, store little if any stable copper when fed toxic levels.

Treatment of Wilson's disease consists of both D-penicillamine and low copper diet therapy. Speech will deteriorate to a per-

ceptibly significant degree if a low copper diet is not followed, even though penicillamine is taken by these patients.² This correlation between a specific defect in speech and normal copper of the diet needs further study to determine if the copper is taken up in ionic form in speech areas of the brain. □

1. C. A. Owen, Jr.: Similarity of Chronic Copper Toxicity in Rats to Copper Deposition of Wilson's Disease. *Mayo Clin. Proc.* 49: 368-376, 1974
2. W. R. Berry, A. E. Aronson, F. L. Darley, and N. P. Goldstein: Effects of Penicillamine Therapy and Low-Copper Diet on Dysarthria in Wilson's Disease (Hepatolenticular Degeneration). *Mayo Clin. Proc.* 49: 405-408, 1974

EFFECT OF (-)-HYDROXYCITRATE ON LIPOGENESIS, APPETITE AND BODY WEIGHT IN RATS

Administration of (-)-Hydroxycitrate (HC), (a competitive inhibitor of the enzyme ATP-citrate lyase) to rats causes marked reduction in in vivo lipogenesis, food intake, body weight gain, and total body lipid. Chronic administration of HC elevates in vitro rates of lipogenesis.

Key Words: lipid, lipogenesis, hepatic, cholesterol, enzyme

(-)-Hydroxycitrate (HC) the principal acid of the fruit rinds of *Garcinia cambogia* has been shown to be a competitive inhibitor of ATP-citrate lyase (EC 4.1.3.8), the enzyme catalyzing the extramitochondrial cleavage of citrate to oxaloacetate and acetyl CoA.¹ Inhibition of this enzymatic reaction should limit the availability of 2-carbon units for fatty acid and cholesterol synthesis.

Indeed A. C. Sullivan and her co-workers² using a meal-fed rat as an animal model geared to elevated lipid synthesis, demonstrated that acute administration of HC inhibits, in a dose dependent manner, in vitro rates of lipogenesis in hepatic cell-free and slice systems, and also in vivo rates of hepatic fatty acid and cholesterol synthesis.

In two recent papers the same group of workers (A. C. Sullivan, J. G. Triscari, J. G. Hamilton, O. N. Miller, and V. R. Wheatley) investigated the effects of chronic administration of HC on lipo-

genesis, weight gain, and appetite in rats.^{3,4} The characteristics of inhibition of lipogenesis after the acute administration of HC were also investigated further.

In vivo rates of lipogenesis were determined by the intravenous administration of ¹⁴C-alanine and ³H-water as precursors of lipids. The latter revealed total fatty acid and cholesterol synthesis independent of the source of carbon precursors of the acetyl groups. Earlier experiments had indicated that ¹⁴C-alanine was equivalent to either ¹⁴C-pyruvate or ¹⁴C-lactate as a carbon precursor for lipogenesis.

Female rats weighing 120 to 160 g were housed under controlled conditions of temperature and duration of light. They were fed a commercial diet (G-70) containing 23 percent casein and 1 percent corn oil, with glucose, cellulose, vitamins, and minerals. Feeding was done once a day between 8 to 11 A.M. (meal feeding). When HC or citrate was added, the equivalent weight of sucrose was deleted from the diet.

Maximum incorporation of label in serum lipids was seen 30 minutes after administration and at that time most of the radioactivity was in the neutral lipid fraction indicating that it was from the liver. Peak incorporation of ^{14}C -alanine in hepatic lipids was also seen at 30 minutes, but that of ^3H -water continued to be linear up to 1 hour. In all subsequent experiments on in vivo lipogenesis the animals were sacrificed 30 minutes after the injection of the labeled compounds.

In the "acute effect" studies, the animals were prefasted for 48 hours, then meal fed the G-70 diet for 6 to 9 days. On the subsequent day HC was given orally in saline directly before giving 8.7 g of food. Control animals received only saline. The animals were sacrificed, at the desired time interval after completion of the meal period, the labeled compounds having been administered 30 minutes before sacrificing.

Hepatic lipogenic rates increased to a maximum at 3 to 5 hours after feeding and declined subsequently to a minimum at 24 hours. Administration of 2.63 mmoles per kilogram of HC, caused significant inhibition of the lipogenic rate for 8 hours after refeeding. During this period, lipid synthesis from ^{14}C -alanine and ^3H -water was reduced by 68 percent and 72 percent respectively. By 12 hours the effect of HC was not apparent, and both the groups had comparable rates of lipogenesis.

Inhibition of lipogenesis due to HC was also apparent in other tissues such as adipose tissue and the small intestine. This was despite markedly varying rates of lipogenesis seen in the three tissues (adipose tissue, liver, small intestine). The anti-lipogenic effect of HC was dose dependent when tested at doses of 2.63, 5.26, and 10.52 mg per kilogram.

In the chronic feeding experiments, HC was administered 1 hour before the meal for a period of 11 to 30 days. Three doses of 2.63, 1.32, and 0.66 mmoles per kilogram were tested. In addition to the study of in vivo lipogenesis, the in vitro rates of hepatic lipogenesis with and without in vitro addition of HC were also examined.

As expected, chronic administration of HC depressed in vivo lipogenesis. The treated rats, however, showed a markedly elevated in vitro rate, indicating greater lipid synthesizing enzymatic potential. Addition of HC in vitro to the reaction mixture diminished the rate of lipogenesis.

The elevation in the in vitro lipid synthesizing capacity might be a compensatory phenomenon to the daily depression of FA synthesis, resulting from the competitive inhibition of ATP citrate lyase. The elevated enzyme could be ATP citrate lyase, since addition of HC in vitro lowered in vitro lipogenesis. Pair feeding showed that the effect of HC on lipogenesis was not due to food restriction.

The fate of unutilized 2-carbon fragments is not clear. The data reported do not support the possibility of a delayed rise in the rate of in vivo lipid synthesis in the treated animals. Acute oral administration of HC was very recently reported to increase the in vivo rate of glycogenesis for several hours after feeding. Treated rats had higher hepatic glycogen. Some of the carbons diverted from fatty acid synthesis are probably channeled into glycogen formation.⁵

Chronic oral administration of HC to growing rats for 11 to 30 days caused a significant reduction in body weight gain, food consumption, and total body lipid. Equimolar amounts of citrate did not have the same effect. In fact, animals treated with citrate showed 35 percent higher weight gain. Liver size and lipid content were not affected with either treatment. Unlike the effect on lipogenic rats, the decrease in weight gain and body lipid was due to lower food intake and not specific for HC. The dose of HC required to produce these changes was lower when the compound was fed in two divided doses rather than a single dose.

These results suggest that HC may be of some value in the control of obesity and treatment of certain lipid disorders, particularly since HC does not enter the mitochondria and hence may not affect normal energy production.² □

1. J. A. Watson, M. Fang, and J. M. Lowenstein: Tricarballoylate and Hydroxycitrate: Substrate and Inhibitor of ATP: Citrate Oxaloacetate Lyase. *Arch. Biochem. Biophys.* 135: 209-217, 1969
2. A. C. Sullivan, J. G. Hamilton, O. N. Miller, and V. R. Wheatley: Inhibition of Lipogenesis in Rat Liver by (-)-Hydroxycitrate. *Arch. Biochem. Biophys.* 150: 183-190, 1972
3. A. C. Sullivan, J. Triscari, J. G. Hamilton, O. N. Miller, and V. R. Wheatley: Effect of (-)-Hydroxycitrate upon the Accumulation of Lipid in the Rat: I. Lipogenesis. *Lipids* 9: 121-128, 1974
4. A. C. Sullivan, J. Triscari, J. G. Hamilton, and O. N. Miller: Effect of (-)-Hydroxycitrate upon the Accumulation of Lipid in the Rat: II: Appetite. *Lipids* 9: 129-134, 1974
5. A. C. Sullivan, J. Triscari, and O. N. Miller: The Influence of (-)-Hydroxycitrate on in vivo Rates of Hepatic Glycogenesis, Lipogenesis and Cholesterogenesis. *Fed. Proc.* 33: 656, 1974

HYPERLIPEMIA AND IRON DEFICIENCY

An iron deficient diet produces anemia and depletion of iron stores in pregnant rats. The offspring, when they are suckled by iron deficient mothers, are also iron deficient and develop significant hyperlipemia.

Key Words: anemia, iron deficiency, iron stores, serum lipids

An association between iron deficiency anemia and lipidemia in animals has been on record for the past 65 years,¹ but the mechanism of the association is still obscure. In most of the original studies, anemia was produced by repeated bleeding. Recently, H. A. Guthrie and her co-workers² examined the effect of iron deficiency in dams on blood lipids in the dams themselves and in their offspring after 18 days of suckling.

Female rats were fed a diet containing 307 or 5 ppm of iron. Some animals were kept on the control high-iron content diet throughout gestation and lactation. A second group was kept on the iron deficient diet throughout gestation and lactation. In two other groups, however, animals were switched from a high to a low iron containing diet at parturition or conversely some were switched from a low to a high iron containing diet at this time.

Some dams were sacrificed at parturition. The iron deficient animals showed no

change in body weight, liver, or spleen weight; but liver iron content was 25 percent of the control value. The hematocrit was also reduced significantly but the fall in total iron-binding capacity and serum iron did not reach the level of statistical significance. In those dams sacrificed 18 days after parturition, the liver iron stores were significantly reduced in the animals on a deficient diet continuously, and also in those who had been on a deficient diet only during lactation. The animals which had switched from a deficient to a normal diet at parturition had restored their tissue iron level to normal. It was of interest that the splenic iron was significantly reduced in any group that had ever been fed the iron deficient diet. The hematocrit and serum iron were significantly low in those animals which had been on a deficient diet during pregnancy and lactation.

The newborn pups from the iron deficient mothers were iron deficient, as judged by reduced liver and spleen iron content. In those sacrificed after 18 days suckling, the changes were varied. The most striking

was a gross reduction in hematocrit and content of iron in the liver and spleen of the pups from dams which were on the iron deficient diet throughout pregnancy and lactation.

These data show that the diet produced significant reduction in iron stores in the pregnant and lactating rat even when the diet was deficient during lactation. This was enough time for an iron deficiency to develop. Similarly, the pups of iron deficient mothers were iron deficient themselves.

The effect of iron deficiency on the serum lipids was of greater importance. Iron deficiency in the dams throughout pregnancy and lactation had no effect on the serum lipids. The pups from those dams which were on the deficient diet throughout pregnancy and lactation were the only ones to show an increase in triglycerides, cholesterol, and phospholipids. In addition their serum electrophoresis pattern had more diffuse pre-beta and alpha-lipoprotein bands than sera from other pups. The authors note an internal correlation between the various lipid fractions, but surprisingly there is no mention of any correlation between the levels of serum lipids and other indices of the anemia or the level of the iron deficiency.

The mechanism of the hyperlipemia is not clear from these studies. Previous workers³ showed that weanling rats on an iron deficient diet became anemic and had elevated triglycerides, but cholesterol and phospholipid levels remained normal. They attributed the hyperlipemia partly to a fall in lipoprotein lipase activity demonstrated in serum and tissue. In a similar study, E. K. Amine and D. M. Hegsted⁴ also showed that iron deficiency in rats and chicks led to hypertriglyceridemia but the level of serum cholesterol remained low. The extent of the triglyceridemia was dependent upon the kind of fat included in the diet. It was high when the diet contained coconut oil but not greatly elevated when

the diet contained unsaturated oils. These results are not entirely consistent with the data presented by Guthrie et al. who utilized a diet containing corn oil. All of the pups, whether fed deficient or control diets, showed elevations in the level of cholesterol while triglycerides were high only in the deficient animals. These differences in response are possibly explained by the degree of anemia. The hematocrit levels in the animals studied by Amine and Hegsted were in the order of 20 percent whereas the most deficient animals studied by Guthrie et al. had an average hematocrit of 13.5 percent.

It should be clear that it is unlikely that iron deficiency is causally related to hyperlipemia in man. Epidemiologic data suggest a contrary relationship and indeed the studies of P. C. Elwood and co-workers⁵ indicated that hypolipemia was associated with low hemoglobin values rather than the reverse. These differences are probably also related to the degree of anemia. Marked aberrations in serum lipid levels in the experimental animals were associated with very severe anemia such as is rarely found in man, at least from uncomplicated iron deficiency. □

-
1. T. R. Boggs and R. S. Morris: Experimental Lipemia in Rabbits. *J. Exp. Med.* 11: 553-560, 1909
 2. H. A. Guthrie, M. Froozani, A. R. Sherman, and G. P. Barron: Hyperlipidemia in Offspring of Iron-Deficient Rats. *J. Nutrition* 104: 1273-1278, 1974
 3. M. Lewis and R. M. Iammarino: Lipemia in Rodent Iron-Deficient Anemia. *J. Lab. Clin. Med.* 78: 546-554, 1971
 4. E. K. Amine and D. M. Hegsted: Iron Deficiency Lipemia in the Rat and Chick. *J. Nutrition* 101: 1575-1582, 1971
 5. P. C. Elwood, R. Mahler, P. Sweetnam, F. Moore, and E. Welsby: Association Between Circulating Haemoglobin Level, Serum-Cholesterol, and Blood Pressure. *Lancet* 1: 589-591, 1970

HORMONAL REGULATION OF FORMIMINOTRANSFERASE

Glucagon and epinephrine administered intravenously lead to a rapid increase in hepatic and jejunal formiminotransferase, whereas insulin produces a decrease in this activity in rat and man.

Key Words: formiminotransferase, folic acid, cyclic AMP, hormonal regulation

Adaptive changes in several rate-limiting enzymes of glycolysis and gluconeogenesis follow dietary intake of carbohydrate¹ or oral folic acid.² Adaptive increases in several jejunal folate-metabolizing enzymes also result from dietary carbohydrate, as well as folate.³ As several hormones that are mediated by cyclic 3',5'-adenosine monophosphate regulate such changes in carbohydrate metabolism,⁴ it became of interest to see if they would have a similar effect on folate metabolism.

F. B. Stifel et al. have now investigated the effects of glucagon, insulin, epinephrine, and cyclic AMP on hepatic and jejunal formiminotransferase activity in rat and man.⁵ This transferase is a key enzyme in the utilization of N-formimino-L-glutamate, derived from histidine catabolism, for formation of 5-formimino-tetrahydrofolate. The latter is ultimately converted to such important compounds as N¹⁰-formyltetrahydrofolate, which is involved in purine biosynthesis. Deficiency of formiminotransferase results in elevated serum folate, formiminoglutamicaciduria, a subtle megaloblastic anemia, and increased urinary aminoimidazolecarboxamide.

In their work, Stifel et al.⁵ used 150 to 230 g male Carworth rats maintained on rat chow and water ad libitum. The animals were anesthetized with pentobarbital administered intraperitoneally, the abdomens opened, and hormones or 0.9 percent saline injected into the portal vein following the removal of control samples of liver or jejunum. In some cases, puromycin dihy-

drochloride (23 mg) or actinomycin D (0.66 μ g per gram) were injected intraperitoneally an hour to two before administration of hormone. In other cases, nucleotides were injected at 5, 10, or 15 minutes prior to removal of liver samples.

Human studies were conducted with five normal male subjects who were fed in six equal portions per day a diet containing in percent: carbohydrate (equal glucose and fructose), 50; corn oil, 30; sodium caseinate, 20. Each person also received 192 mg of ferrous sulfate and one nonavitamin tablet daily. The folic acid content was 14 μ g. Jejunal biopsies were obtained on days four and nine. Before the first feeding on day six, 15 units of NPH insulin was injected subcutaneously. Other studies included four children, two of whom had glucose-6-phosphatase deficiency, and the other two debrancher (amylo-1,6-glucosidase) deficiency. From these patients, liver biopsies were obtained before and after 2 or 3 minutes of intravenous infusion of glucagon (1 mg per minute).

Formiminotransferase was measured spectrophotometrically at 355 nm essentially as described by T. Arakawa et al.⁶ on the supernatant solution (60 minutes at 104,000 \times gravity) from homogenates of liver or jejunal mucosa. Formimino-L-glutamate was assayed according to H. Tabor and L. Wyngarden.⁷ Cyclic AMP was quantitated in liver following the method of A. G. Gilman⁸ and with the addition of tritium-labeled compound to monitor recovery.

A significant ($P < 0.01$) increase in hepatic formiminotransferase was found 15

minutes after as little as 1.5 μ g of glucagon administered to rats. Maximal response occurred with 100 or more times this level of the hormone, which caused enzyme activity in both liver and jejunal mucosa to nearly double, while the level of hepatic formiminoglutamate fell correspondingly. Pretreatment of animals with puromycin or actinomycin D to inhibit protein (new enzyme) synthesis had no effect. Insulin elicited the opposite effect, viz. a decrease in formiminotransferase activity, which was significant ($P < 0.01$) with as little as 0.015 units per kilogram and was nearly maximal with ten times more than this. The hepatic formiminoglutamate increased in inverse correspondence. Again, puromycin and actinomycin D failed to block the effect. Infusion of epinephrine (1 to 2 μ g per minute) produced a rapid stimulatory response on the hepatic transferase and rise in level of cyclic AMP. When 50 μ moles of nucleotide was similarly administered, a small, but significant ($P < 0.01$) increase in the transferase activity was found with cyclic AMP, whereas no effect was detected with cyclic GMP, 5'-AMP, or ATP.

Although less thoroughly studied, the same general effects on hepatic and jejunal formiminotransferase appear to follow the administration of the hormones to humans. Intravenous glucagon (1 mg per minute) approximately doubled the level of the hepatic transferase of the four child patients within two to three minutes. Subcutaneous insulin (15 units per each of three days) decreased the jejunal transferase activity of the five normal adults fed the high carbohydrate diet.

Stifel et al.⁵ point out that their demonstration of the acute reciprocal hormonal regulation of the formiminotransferase by insulin and glucagon resembles the changes found in the regulation of the gluconeogenic fructose diphosphatase by the same hormones.⁹ The investigators speculate that the control of transferase mediated by cyclic AMP may involve a dephosphorylation-phosphorylation mechanism analogous to that involved in the regulation of glycogen metabolism. In any event, the

hormonal influence on formiminotransferase may well be at the enzyme alteration level, since inhibitors of protein biosynthetic machinery have no effect. The relationship between folate and carbohydrate metabolism may also exist at several levels of operation. \square

1. M. C. Scrutton and M. F. Utter: The Regulation of Glycolysis and Gluconeogenesis in Animal Tissues. *Ann. Rev. Biochem.* 37: 249-302, 1968
2. R. H. Herman, F. B. Stifel, Y. F. Herman, and N. S. Rosensweig: The Response of Jejunal Glycolytic Enzymes to a Folate Deficient Diet in Germ-Free and Pathogen-Free Rats. *Fed. Proc.* 28: 628, 1969
3. F. B. Stifel, R. H. Herman, and N. S. Rosensweig: Dietary Regulation of Glycolytic Enzymes. VII. Effect of Diet and Oral Folate Upon Folate-Metabolizing Enzymes in Rat Jejunum. *Biochim. Biophys. Acta* 208: 381-386, 1970
4. J. P. Jost and H. V. Rickenberg: Cyclic AMP. *Ann. Rev. Biochem.* 40: 741-774, 1971
5. F. B. Stifel, O. D. Taunton, H. L. Green, E. G. Lufkin, L. Hagler, and R. H. Herman: Hormonal Regulation of Hepatic and Jejunal Formiminotransferase Activity in Man and Rat. *Biochim. Biophys. Acta* 354: 194-205, 1974
6. T. Arakawa, K. Narisawa, K. Tanno, K. Hara, O. Higashi, Y. Honda, T. Tamura, Y. Wada, T. Mizuno, and T. Hayashi: Megaloblastic Anemia and Mental Retardation associated with Hyperfolic-Acidemia: Probably due to N⁵ Methyltetrahydrofolate Transferase Deficiency. *Tohoku J. Exp. Med.* 93: 1-22, 1967
7. H. Tabor and L. Wyngarden: The Enzymatic Formation of Formiminotetrahydrofolic Acid, 5,10-Methenyltetrahydrofolic Acid, and 10-Formyltetrahydrofolic Acid in the Metabolism of Formiminoglutamic Acid. *J. Biol. Chem.* 234: 1830-1846, 1959
8. A. G. Gilman: A Protein Binding Assay for Adenosine 3':5'-Cyclic Monophosphate. *Proc. Nat. Acad. Sci. USA* 67: 305-312, 1970
9. O. D. Taunton, F. B. Stifel, L. H. Greene, and R. H. Herman: Rapid Reciprocal Changes of Rat Hepatic Glycolytic Enzymes and Fructose-1,6-diphosphatase Following Glucagon and Insulin Injection In Vivo. *Biochem. Biophys. Res. Commun.* 48: 1663-1670, 1972

ELEVATED XANTHINE OXIDASE IN VITAMIN E DEFICIENCY

Immunochemical evidence is presented that the increased xanthine oxidase activity that occurs in the livers of rabbits deficient in vitamin E is due to an increased accumulation of the enzyme protein.

Key Words: vitamin E, xanthine oxidase

An observation that vitamin E deficiency in rabbits and monkeys led to a marked increase in allantoin excretion prompted J. S. Dinning¹ to investigate liver xanthine oxidase and uricase levels in this deficiency. A marked increase in liver xanthine oxidase was found to occur while uricase activity was normal. The increased activity of xanthine oxidase was not affected by the in vitro addition of either α -tocopherol phosphate or normal liver homogenates. Further studies by G. L. Catignani and J. S. Dinning² tended to rule out the possibility that an activation of preformed xanthine oxidase was occurring in vitamin E deficiency and these authors suggested that vitamin E regulates the synthesis of the enzyme. Evidence has now been presented by G. L. Catignani and his co-workers³ that an accelerated de novo synthesis probably occurs in vitamin E deficiency in rabbits.

These recent studies of Catignani et al. were made possible by the ability of the authors to make a preparation of xanthine oxidase from the livers of rabbits deficient in vitamin E which showed homogeneity on acrylamide gel electrophoresis (unpublished results). Antibodies to the enzyme were obtained by injecting the enzyme into a sheep. Immuno-electrophoresis of the antibody preparation showed a single precipitin arc in the presence of the purified xanthine oxidase. Livers from normal and vitamin E deficient rabbits were homogenized and centrifuged at 105,000 x-g. Increasing amounts of both supernatants

(cytosol) were then incubated in the presence of a fixed amount of either control or immune serum, and the xanthine oxidase activity measured. A plot of activity versus the amount of cytosol yielded an equivalence point, the amount of cytosol necessary to furnish sufficient xanthine oxidase to react with the fixed amount of antibody that was employed. A series of such experiments of immunochemical titration revealed that the cytosol prepared from livers of vitamin E deficient rabbits had four to nine times the capacity to neutralize the enzyme antibody as that from normal animals. The enzyme activity of the cytosol prepared from the experimental animals was also four to nine times that obtained from the normal animals. A graph is presented by the authors in which the activity of original cytosol is plotted against the activity found in the presence of the fixed amount of antibody. Such a graph reveals identical lines for both normal and experimental cytosol and demonstrates that the xanthine oxidase which accumulates during vitamin E deficiency is immunologically indistinguishable from that in the normal animal.

A second approach to the problem is made by measuring the incorporation of radioactive leucine into xanthine oxidase. Control and vitamin E deficient rabbits were fasted overnight and ¹⁴C-leucine was injected. Six hours later the animals were sacrificed and liver cytosols prepared. From these cytosols the following fractions were made and analyzed for radioactivity: immunoprecipitable protein, total protein

as precipitated by trichloroacetic acid, and leucine isolated from the trichloroacetic acid supernatants. The radioactivity of the immunoprecipitable protein obtained from the deficient animals was 357 and 821 percent higher than that of the control animals. In these experiments the enzyme activity of the cytosol from the deficient animals was 670 and 890 percent of the control value. No significant difference was observed between experimental and control animals in the radioactivity of the protein precipitated from the cytosol by trichloroacetic acid. The specific activity of the leucine isolated from the trichloroacetic acid supernatant of the cytosol from the deficient rabbits was 40 percent lower than that from the control animal. This latter finding of an apparent "decreased availability" of labeled leucine in the deficient animals is interpreted by the authors to substantiate "the concept that an elevated rate of synthesis of the enzyme occurs during vitamin E deficiency". It is puzzling that this decrease in the pool of labeled leucine is not reflected in the radioactivity of the protein precipitated by trichloroacetic acid from the liver cytosol of the deficient animals.

The authors make the reasonable conclusion that their results show that the mechanism of increase of liver xanthine oxidase in vitamin E deficient rabbits involves the accumulation of the enzyme protein rather than an activation of the enzyme. They favor the interpretation that the accumulation of xanthine oxidase reflects an accelerated de novo synthesis of the enzyme. They are careful, however, to

point out that a part of the accumulation of the enzyme may be due to inhibition of its degradation. In any case their results seem to indicate clearly the direction that future investigations must follow in elucidating the mechanism responsible for this change. One possibility suggested by the authors is that in vitamin E deficiency there is an alteration of the transcription of the messenger RNA for xanthine oxidase. Earlier workers^{1,2} suggested that vitamin E somehow regulates the synthesis of the enzyme. The possibility must not be overlooked that the increase in xanthine oxidase may be secondary to a more primary aberration in nucleic acid metabolism as suggested by Dinning.¹ Thus the increase in xanthine oxidase might be a reflection of an increase in the flux of its substrates and resemble the marked changes in liver glucose-6-phosphate dehydrogenase activity that occurs with alterations in dietary carbohydrate intake. The relationship between xanthine oxidase activity and vitamin E levels certainly seems to be worthy of more intensive study. □

1. J. S. Dinning: An Elevated Xanthine Oxidase in Livers of Vitamin E Deficient Rabbits. *J. Biol. Chem.* 202: 213-215, 1953
2. G. L. Catignani, Jr. and J. S. Dinning: Role of Vitamin E in the Regulation of Rabbit Liver Xanthine Oxidase Dehydrogenase Activity. *J. Nutrition* 101: 1327-1330, 1971
3. G. L. Catignani, F. Chytil, and W. J. Darby: Vitamin E Deficiency: Immunochemical Evidence for Increased Accumulation of Liver Xanthine Oxidase. *Proc. Nat. Acad. Sci. USA* 71: 1966-1968, 1974

HAZARDS OF OVERUSE OF VITAMIN D

*A Statement of the FOOD AND NUTRITION BOARD,
NATIONAL ACADEMY OF SCIENCES, NATIONAL RESEARCH COUNCIL*

Prepared by the Committee on Nutritional Misinformation

An excess intake of vitamin D can result in serious toxicity. Vitamin D is stored in the fatty tissues of the body and is present in the circulating plasma. Because vitamin D promotes absorption of calcium from the intestine, a large excess of stored vitamin D can cause excessive quantities of calcium in the blood (hypercalcemia) persisting for months after intake of vitamin D has been discontinued. Chronic hypercalcemia causes calcification of soft tissues with particularly serious injury to the kidney; associated general symptoms are weakness, lethargy, anorexia, and constipation. The sensitivity of individuals to an excess of vitamin D is quite variable so that it is not possible to state the minimal toxic dose. Overuse of vitamin D in England and the European continent during the 1940's and 1950's is thought to be the cause of a serious disorder of infancy called "idiopathic hypercalcemia" that was seen with unusual frequency in that period. Following reduction of vitamin D intake to levels approximating those considered adequate in this country, "idiopathic hypercalcemia" has become quite rare.

Vitamin D is an unusual nutrient in that its major natural source is not food, but rather the 7-dehydrocholesterol in the skin, which is converted to vitamin D by the short wave ultraviolet component of sunshine. The usual foods of infants including breast milk contain little vitamin D. Without exposure to sunshine or fortifica-

tion of the diet with vitamin D, vitamin D deficiency results in infants. In some industrial cities of the temperate zones infants may not get sufficient exposure to ultraviolet light because of the combination of climatic conditions and atmospheric smog. Smog absorbs most of the sun's short wave ultraviolet light radiation even on sunny days. For this reason rickets, the disease resulting from defective mineralization of bone due to lack of vitamin D, was once extremely common in infants and children in northern Europe and the United States. Because of the widespread use of vitamin D-fortified milk and infant feeding preparations, rickets has become an exceedingly rare disease in this country.

The vitamin D requirement of infants during the rapidly growing period of the first six months of life, can, and has been, accurately determined. In this age period a daily intake of 400 I.U. of vitamin D is adequate with an ample margin of safety for normal biological variation. For most infants 100 I.U. per day in milk would probably suffice. Vitamin D is also required by older children and adults but determination of the true requirement beyond infancy is extremely difficult, and the assumption has been made that a daily intake of 400 units meets the needs beyond infancy as well. This seems justified by our present experience. In the adult, the poor mineralization of bone resulting from vitamin D deficiency is termed osteomalacia.

Nutritional osteomalacia due to lack of vitamin D has been described particularly in elderly patients on highly restricted diets estimated to provide less than 100 I.U. of vitamin D per day. The normal child, the adult, and the pregnant or lactating woman do not require more than 400 I.U. of vitamin D per day. These normal requirements are met by exposure to sunshine and consumption of such foods as vitamin D-fortified milk, egg yolk, and fish, such as salmon, sardines, herring, and tuna. The use of vitamin D concentrates is necessary for breast fed infants and infants on non-fortified milk but is rarely required for the proper vitamin D nutrition of other infants, children, or adults.

Use of highly concentrated preparations of vitamin D may be required by patients with specific diseases requiring unusual amounts of vitamin D. This treatment must be closely supervised by a physician. Vitamin D must be metabolized in the liver and kidney before it becomes the active compound that regulates the calcium and phosphate metabolism of the body and ensures normal bone mineralization. The vitamin D requirements of patients with liver disease and kidney disease must be

separately determined and such patients may require much greater amounts of this vitamin than is normally given. Because of poor absorption of vitamin D by patients with intestinal malabsorption increased amounts of dietary vitamin D are needed in their treatment.

In summary, excessive amounts of vitamin D are hazardous and only individuals with diseases affecting vitamin D absorption or metabolism require more than 400 I.U. per day. Such needs should be established by clinical evaluation, and treatment should be specifically recommended and supervised by physicians. □

American Academy of Pediatrics Committee on Nutrition: The relation between infantile hypercalcemia and vitamin D-public health implications in North America. *Pediatrics* 40: 1050, 1967

Gough, K. R., Lloyd, O. C., and Willis, M. R.: Nutritional Osteomalacia, *Lancet* II: 1261, 1964

Harrison, H. E.: *Calcium Metabolism in Pediatrics*, 15th Edition, H. L. Barnett, ed., Appleton-Century-Crofts, New York, 1972

Copies of this report may be obtained from NATIONAL ACADEMY OF SCIENCES, Printing and Publishing Office, 2101 Constitution Avenue, Washington, D. C. 20418. Requests should be accompanied by a self-addressed, stamped envelope.

NUTRITION NOTES

Meeting Announcement

The Xth International Congress of Nutrition (XICN), sponsored by the International Union of Nutritional Sciences (IUNS), will be held in Kyoto, Japan, from August 3 through August 9, 1975, at the Kyoto International Conference Hall (KICH). Previous Congresses took place in Hamburg (1966), Prague (1969) and Mexico City (1972). Japan was designated as the site of the Xth Congress at the Mexico Congress.

The Science Council of Japan, an adhering body to the IUNS, will be the host of the Kyoto Congress with the support of the Japanese Government. The Congress will be open to all interested persons and is expected to draw more than 2,500 experts in nutritional sciences and related areas from all over the world.

Organizers of the International Congress hope that many scientists from this country who engage in the nutritional sciences and their application will participate in the Congress, and that they, aiming at our symbolic theme "Through Science and Nutrition to Human Wellbeing", might be able to enhance better understanding and exchange with foreign scholars.

Congress Schedule

The Congress will open with a ceremony to be held in the Main Hall of the Kyoto International Conference Hall on Sunday morning, August 3, 1975. The ceremony will be followed the same afternoon by a special symposium on "World Food Needs and Resources — the Current Crisis and Future Prospects."

The Congress will close with a ceremony which will take place on Saturday afternoon, August 9. During the intervening period, all Congress sessions will be held in the KICH from 9:00 to 12:00 and from 13:30 to 17:00.

The ordinary technical sessions will consist of symposia and free communication

sessions. Six to seven symposia will be held simultaneously every morning and there will be several free communication sessions on four afternoons.

The symposia will be held on seven subjects. In each session, three to six papers, recruited by invitation only, will be presented. The audience will have the opportunity and is encouraged to participate in these discussions.

Submissions of Abstracts

Each active member is entitled to submit one abstract for free communications by using the special Form A and B enclosed in the Second Circular Book of the Xth ICN. Abstracts must be received before the deadline of February 28, 1975. Abstracts will be accepted only from those who have paid the registration fee for active membership by April 30, 1975. Authors will be notified by May 31, 1975 of the acceptance of their abstracts for oral presentation.

Registration Fee

Active Member: US\$ 85.00 or ¥ 29,000 (per person)

Affiliate Member: US\$ 40.00 or ¥ 10,000 (per person)

Deadline: Registration with payment is requested to be completed not later than April 30, 1975 (post-marked).

Late Fee: After the deadline has passed, participants seeking to register are asked to pay an additional US\$ 10.00 or ¥ 3,000 per person (both Active and Affiliate Members).

Correspondence

All correspondence related to the Congress should be addressed to:

Secretariat, XTH INTERNATIONAL
CONGRESS OF NUTRITION

c/o Kyoto International Conference Hall
Takaraike, Sakyo-ku, Kyoto, 606 Japan
Phone: 075-791-3111

Cable: INTHALL KYOTO

Current Status of AMA Council on Foods and Nutrition

According to the Board of Trustees of the American Medical Association, the Council on Foods and Nutrition is not terminated but continues to exist on an inactive status,

pending the action of the House of Delegates at the American Medical Association Annual Meeting. □

New

Unique

INDEX OF NUTRITION EDUCATION MATERIALS

The Index of Nutrition Education Materials has been prepared by the Office of Education of the Nutrition Foundation to assist teachers and the general public in acquiring useful teaching aids and educational materials.

The Index lists more than 1,000 booklets, pamphlets, audio-visual aids and lists of recommended books available from federal agencies, professional societies, and educational organizations or foundations. Other sources for education material are included.

Gives addresses of important local contacts and sources in each state.

Source, subject matter, readership level and description are given for each publication in color-coded sections for easy reference. \$5.00 postpaid

Order from:

OFFICE OF EDUCATION
THE NUTRITION FOUNDATION
888 Seventeenth Street, N.W.
Washington, D. C. 20006

Cobalt

by Eric J. Underwood, Ph.D.



The first evidence that cobalt is a dietary essential was obtained just 50 years ago as an outcome of Australian researches into the cause of two naturally occurring debilitating diseases of sheep and cattle known locally as 'coast disease' and 'wasting disease'.^{1,2} Prior to this, workers in New Zealand had shown that a similar disease of cattle, known as 'bush-sickness', could be cured and prevented by the oral administration of large amounts of crude iron salts and ores. Iron deficiency then became accepted as the cause of this and similar diseases, until J. F. Filmer and E. J. Underwood,³ who had become suspicious of the large amounts of iron compounds required to cure wasting disease, prepared an iron-free extract of one of these curative compounds and found it to be just as potent as the whole compound. This led to the hypothesis that the disease was due to a deficiency in the soils and herbage of the affected areas of some trace element which occurred as a contaminant of the iron compounds successfully used. The trace element was subsequently shown to be

cobalt² and normal growth and health of sheep and cattle were secured by the administration of small (0.1 mg to 1.0 mg Co per day) oral doses of a cobalt salt.⁴

During the course of these investigations it was found that whole liver administered orally was also curative of wasting disease. Liver was tested in this way because it had just been shown to control pernicious anemia in man and anemia was a common manifestation of the disease of cattle under study. Liver ash administered in comparable doses to whole liver was ineffective. This led to the suggestion that the potency of liver was due to the presence of a stored factor and that cobalt functions through the production of this factor within the body.⁴ Eleven years were to pass before this hypothesis was validated and the 'stored factor' was shown to be the cobalt-containing vitamin B₁₂.^{5,6} Three years later S. E. Smith and co-workers⁷ at Cornell effected complete remission of all signs of cobalt deficiency in lambs by injections of vitamin B₁₂. Cobalt deficiency in ruminants then emerged as a vitamin B₁₂ deficiency brought about by the inability of the rumen micro-organisms, in the presence of inadequate dietary cobalt, to synthesize sufficient vitamin B₁₂ to meet the needs of the host animal's tissues for this vitamin. A unique nutritional situation was thus disclosed; a situation in which animals utilize a trace element solely as an integral part of a vitamin and are completely dependent upon the symbiotic activities of their gastrointestinal micro-organisms for their supply of that vitamin. The cobalt status of ruminant diets is therefore crucial to their growth and health.

Dr. Underwood, formerly Director, Institute of Agriculture, University of Western Australia, is at present Chairman, National Committee on Nutritional Sciences, Australian Academy of Science and a Member of the Executive of the Commonwealth Scientific and Industrial Research Organization (Australia), Private Bag, P. O., Wembley Western Australia 6014, Australia.

A limited number of reprints of this article may be obtained from the author. THERE ARE NO REPRINTS OF UNSIGNED REVIEWS.

A further unique aspect of cobalt is that it must be supplied in the diet of man and other monogastric species entirely in its physiologically-active form, cobalamin or vitamin B₁₂. The tissues of these species are unable to synthesize the vitamin from dietary cobalt and their intestinal microflora have an extremely limited capacity to effect this vital transformation at a point in the digestive tract where the vitamin can be absorbed. For all other elements, except possibly chromium, man and animal species with comparable digestive and absorptive processes possess an apparently complete capacity to produce the required physiologically active organic combinations from simple elemental or inorganic forms of the element supplied in the diet. In these circumstances the cobalt status of human foods and dietaries is relatively unimportant — it is their vitamin B₁₂ status that is critical. Furthermore, since 1 µg vitamin B₁₂ per day, as H. A. Schroeder et al.⁸ have said 'can make the difference between life and death from human pernicious anemia' and since vitamin B₁₂ contains 4.34 percent Co, it could be argued that the daily human requirement for cobalt, provided it is all in this form, is an infinitesimal 0.0434 µg.

Cobalt in Foods and Dietaries

The limited data available for the cobalt content of human foods and total diets are extremely variable. Some of this variation undoubtedly stems from analytical errors and some is due to genuine variations arising from soil and climatic differences directly affecting the cobalt content of the foods of plant origin and indirectly affecting those of animal origin. Thus G. K. Murthy et al.¹² in an extensive study of the diets of children from 28 widely separated institutions in the United States, found the cobalt concentration in the total diets to vary from 0.25 to 0.69 mg per kilogram and the total intakes to range from 0.30 to 1.77 mg Co per day, with a mean of 1.02 mg per day. These levels are much higher than the 0.16 to 0.17 mg Co per day for adults consuming North American diets

estimated by I. H. Tipton et al.⁹ or the 0.14 to 0.58 mg Co per day estimated by Schroeder et al.⁸ Such intakes would give cobalt concentrations in the total diet close to 0.2 to 0.5 ppm (dry basis) which is two to five times the cobalt concentration in pastures and fodders found to be just adequate for sheep and cattle.^{4,10,11}

Among individual types of foods the green leafy vegetables are the richest and most variable in cobalt content, while dairy products, refined cereals, and sugar are the poorest. Typical values for the former group are 0.2 to 0.6 ppm Co (dry basis) and for the latter 0.01 to 0.03 ppm Co (dry basis). Normal cow's milk is very low in cobalt with levels ranging from 0.4 to 1.1 µg per liter and with most values lying close to 0.5 µg per liter.¹³⁻¹⁵ The organ meats, liver and kidney, commonly contain 0.15 to 0.25 ppm Co (d.b.) and the muscle meats approximately half those levels. These foods contain much more cobalt than can be accounted for as vitamin B₁₂, although the liver as the main storage organ of the body is a relatively rich source of this vitamin. Fruits, vegetables, and cereals contain none of their cobalt in the form of vitamin B₁₂.

Cobalt Metabolism

Studies of the absorption and excretion of cobalt reveal a rather confusing situation. Several of the early investigations, using radioactive cobalt, indicated that dietary or orally administered cobalt is poorly absorbed by rats and farm animals and is excreted mainly in the feces.^{16,17} R. W. Engel and co-workers¹⁸ also reported that preadolescent girls excreted 90 percent of their total cobalt excretion in the feces and 10 percent in the urine. A very different picture emerges from later studies indicating that cobalt is well-absorbed,²¹ that the major route of excretion is the urine,^{8,9,41} and that there is a direct relationship between the proportion of an oral dose that is absorbed from the intestine and the proportion that is excreted in the urine.^{40,41} It seems that in man only small amounts of cobalt are lost

by way of the feces,^{8,9,40} sweat,⁴² and hair.⁸ It appears, further, that cobalt shares with iron at least part of the same intestinal mucosal transport pathway in which acceleration of transport of both elements is governed by the same mechanism. Thus cobalt absorption, as well as iron absorption, is significantly enhanced in iron-deficient rats,⁴³ in iron deficiency in man,^{40,41} and in patients with portal cirrhosis with iron overload and in those with idiopathic hemochromatosis.⁴¹ The concept that the intestinal transport of cobalt and iron involves a common pathway is compatible with the demonstration of a mutual antagonism between the two elements at the absorptive level, referred to later in connection with cobalt toxicity.²³ Why man and the rat should absorb far more cobalt than they can possibly use is unknown, unless a function for this element exists, in addition to its role in vitamin B₁₂, which has yet to be discovered.

Using neutron activation analysis N. Yamagata et al.¹⁹ analyzed human tissues for cobalt and calculated the whole body content of an average adult man as 1.1 mg Co. On the basis of meager data it seems that cobalt does not accumulate significantly in human tissues with age,^{8,20} as does cadmium in the industrialized world, and does not decrease significantly with age as is common with chromium in individuals consuming western-style diets high in refined and processed foods.

Cobalt Toxicity

Cobalt has a low order of toxicity in all species studied, including man. Daily doses of 3 mg Co per kilogram of bodyweight, which approximates 150 ppm Co in the dry diet (or some 1000 times normal levels) can be tolerated by sheep for many weeks without toxic effects.²² With doses of 4 mg Co per kilogram of bodyweight or higher, appetite and bodyweight are severely depressed, the animals become anemic, and some die. The anemia perhaps arises from a depression in iron uptake by the very high intakes of cobalt. Thus the absorption of ⁵⁹Fe from

jejunal loops of the rat has been shown to be reduced by almost two-thirds in the presence of a ten-fold higher cobalt concentration. A 100-fold excess of cobalt will suppress absorption of ⁵⁹Fe nearly completely.²³ On the other hand, rats and other species, other than the adult ruminant, fed large amounts of cobalt as cobalt salts develop a true polycythemia accompanied by hyperplasia of the bone marrow, reticulocytosis, and increased blood volume.²⁴ The oral intakes of cobalt necessary to produce significant polycythemia, approximately 200 to 250 ppm Co of the total diet, and are therefore clearly many times greater than those that could conceivably be obtained from normal foods and beverages. Cobalt has been reported to stimulate production of erythropoietin,^{31,32} but the amounts required are so large that the effect probably results from tissue hypoxia.³³

The polycythemic effect of cobalt led to the use of cobalt as a non-specific erythropoietic stimulant in man. Cobalt salts have been used in the treatment of the anemia of nephritis and infection²⁵ and several reports have appeared of hemopoietic response to cobalt, in addition to iron, in children and in pregnant women.²⁶⁻²⁸ The amounts required to elicit these responses are so large (20 to 30 mg Co per day) that serious toxic manifestations, including thyroid hyperplasia, myxedema, and congestive heart failure in infants, can occur.^{29,30} Cobalt, therefore, occupies a very restricted place in the management of human anemias.

In certain circumstances, as yet unexplained, cobalt intakes substantially lower than the 20 to 30 mg per day mentioned in the preceding paragraph, can be toxic to man. Cobalt has been incriminated as the precipitating factor in several outbreaks of severe cardiac failure in heavy beer drinkers. Cobalt was suspected because of the high incidence of polycythemia, thyroid epithelial hyperplasia, and colloid depletion noted in the fatalities, in addition to the congestive heart failure.³⁴ Cobalt salts had been added to the beer, a practice no longer in use, to improve its foaming

qualities at concentrations of 1.2 to 1.5 ppm Co. At such concentrations the consumption of 24 pints daily would supply about 8 mg of cobalt sulfate, an amount well below that which can be taken with impunity by normal individuals. In fact, up to 300 mg daily of cobalt salts have been used therapeutically without cardiotoxic effects. It seems that high cobalt and high alcohol intakes are both necessary to induce the distinctive cardiomyopathy, plus a third factor which may be low dietary protein,³⁵ or thiamin deficiency.³⁶ In a recent study of the effect of cobalt, beer, and thiamin-deficiency in pigs, a comparable cardiomyopathy was not observed.³⁷

Cobalt and the Thyroid

Cobalt, and also manganese, have been reported to be necessary for the synthesis of the thyroid hormone in rats.³⁸ The addition of physiological doses of cobalt to a diet which was not naturally high in iodine or cobalt did not affect the weight of the gland, but caused definite histological changes, including a decrease in the size of the follicles and an increase in the height of the epithelial cells. It was concluded that 'the appearance of endemic disturbances of thyroid gland function in people inhabiting biogeochemical provinces with a low iodine and a low cobalt content depend not only on the level of I and Co, but also on the ratio of these elements in the environment'. Other workers in the Soviet Union³⁹ have observed an inverse correlation between the cobalt levels in the foods, waters, and soils in certain areas and the incidence of goiter in man and farm animals. A possible relationship between the cobalt status of the environment and the incidence of goiter warrants investigation in other areas and further critical examination of the suggestive cobalt iodine interaction in animals is desirable. □

1. H. R. Marston, *J. Council Sci. Indian Res. (Australia)* 8: 111-116, 1935, and E. W. Lines, *J. Council Sci. Indian Res. (Australia)* 8: 117-119, 1935

2. E. J. Underwood and J. F. Filmer, *Aust. Vet. J.* 11: 84-92, 1935
3. J. F. Filmer and E. J. Underwood, *Aust. Vet. J.* 10: 83-87, 1934
4. J. F. Filmer and E. J. Underwood, *Aust. Vet. J.* 13: 57-64, 1937
5. E. L. Rickes, N. G. Brink, F. R. Koniusky, T. R. Wood, and K. Folkers, *Science* 108: 134, 1948
6. E. L. Smith, *Nature (London)* 162: 144-145, 1948
7. S. E. Smith, B. A. Koch, and K. L. Turk, *J. Nutrition* 44: 455-464, 1951
8. H. A. Schroeder, A. P. Nason, and I. H. Tipton, *J. Chronic Dis.* 20: 869-890, 1967
9. I. H. Tipton, P. L. Stewart, and P. G. Martin, *Health Phys.* 12: 1683-1689, 1966
10. E. D. Andrews, *New Zealand J. Agr. Res.* 8: 788-817, 1965
11. H. J. Lee and H. R. Marston, *Aust. J. Agr. Res.* 20: 905-918, 1969
12. G. K. Murthy, U. Rhea, and J. T. Peeler, *Environ. Sci. Technol.* 5: 436-442, 1971
13. J. G. Archibald, *J. Dairy Sci.* 30: 293-297, 1947
14. G. H. Ellis and J. F. Thompson, *Indian Eng. Chem. Anal. Ed.* 17: 254-257, 1945
15. M. Kirchgessner, *Z. Tierphysiol. Tierernahr. Futtermittelk* 14: 270-278, 1959
16. C. L. Comar, G. K. Davis, and R. F. Taylor, *Arch. Biochem.* 9: 149-158, 1946
17. C. L. Comar and G. K. Davis, *Arch. Biochem.* 12: 257-266, 1947
18. R. W. Engel, N. O. Price, and R. F. Miller, *J. Nutrition* 92: 197-204, 1967
19. N. Yamagata, S. Muratan, and T. Morii, *J. Radiat. Res. (Tokyo)* 3: 4-8, 1962
20. I. H. Tipton and M. J. Cook, *Health Phys.* 9: 103-145, 1963
21. P. P. Toskes, G. W. Smith, and M. E. Conrads, *Am. J. Clin. Nutrition* 26: 435-437, 1973
22. D. E. Becker and S. E. Smith, *J. Animal Sci.* 10: 266-271, 1951
23. W. Forth and R. Rummel in *Intestinal Absorption of Metal Ions, Trace Elements and Radionuclides*. S. C. Skoryna and Wadron-Edward, Editors, pp. 173-191. Pergamon Press, Montreal, 1971
24. W. C. Grant and W. S. Root, *Physiol. Rev.* 32: 449-498, 1952
25. F. H. Gardner, *J. Lab. Clin. Med.* 41: 56-64, 1953
26. B. L. Coles, *Arch. Dis. Child.* 30: 121-126, 1955
27. F. Tevetoglu, *J. Pediat.* 49: 46-55, 1956

28. H. G. Hamilton, *South. Med. J.* 49: 1056-1060, 1956
29. T. Sederholm, K. Kouvalainen, and B. A. Lamberg, *Acta Med. Scandinav.* 184: 301-306, 1968
30. T. C. Washburn and E. Kaplan, *Clin. Paediat.* (Philadelphia) 3: 89-92, 1964
31. E. Goldwasser, L. O. Jacobson, W. Fried, and L. F. Plzak, *Blood* 13: 55-60, 1958
32. J. W. Fisher and B. J. Birdwell, *Acta Haematol.* (Basel) 26: 224-232, 1961
33. J. W. Fisher and J. W. Langston, *Blood* 29: 114-125, 1967
34. *Nutrition Reviews* 26: 173-175, 1968
35. C. S. Alexander, *Ann. Int. Med.* 70: 411-413, 1969
36. H. T. Grinvalsky and D. M. Fitch, *Ann. N.Y. Acad. Sci.* 156: 544-565, 1969
37. R. E. Burch, R. V. Williams, and J. F. Sullivan, *Am. J. Clin. Nutrition* 26: 403-408, 1973
38. R. I. Blokhima in *Trace Element Metabolism in Animals*. C. F. Mills, Editor, pp. 426-432. S. Livingstone, Edinburgh, 1970
39. V. V. Kovalsky in *Trace Element Metabolism in Animals*. C. F. Mills, Editor, pp. 385-397. S. Livingstone, Edinburgh, 1970
40. L. S. Valberg, J. Ludwig, and D. Olatunbosun, *Gastroenterology* 56: 241-251, 1969
41. L. S. Vallberg in *Intestinal Absorption of Metal Ions, Trace Elements and Radio-nuclides*. S. C. Skoryna and Waldron-Edward, Editors, pp. 257-263. Pergamon Press, Montreal, 1971
42. C. F. Consolazio, R. A. Nelson, L. O. Matoush, R. C. Hughes, and P. Urone, *U.S. Army Med. Res. Nutrition Lab. Rept.* 1-13, 1964
43. S. Pollack, J. N. George, R. C. Reba, R. M. Kaufman, and W. H. Crosby, *J. Clin. Invest.* 44: 1470-1473, 1965

Nutrition Reviews

authoritative articles by distinguished international scientists • competent, critical, and knowledgeable reviews • interpretative summaries of important original clinical and experimental research • selected excerpts from classic publications marking milestones in nutrition research • informative special reports on government and organization actions • contributing editors review journals of widespread interest covering biochemistry, food science, public health, toxicology, dentistry, pediatrics, internal medicine, gastroenterology and other clinical specialties •

Send subscription and renewal requests to:

THE NUTRITION FOUNDATION
489 Fifth Avenue
New York, New York 10017

\$12.00 per year

\$6.00 for students

DIETARY FIBER AND COLONIC FUNCTION — AN EFFECT OF PARTICLE SIZE?

Studies of the effects of two types of bran preparation on colonic function indicated that particle size had an important effect on motility.

Key Words: dietary fiber, bran, water-binding capacity

In the last two or three years there has been a reawakening of interest in clinical nutrition circles concerning the possible role of those dietary constituents derived from the structural components of the plant cell wall or the fiber content of the diet. This increasing interest has its origins in the paper by N. S. Painter and D. P. Burkitt¹ in which they suggested that diverticular disease was the result of a deficiency of 'fiber' in the diet. This view was based on epidemiological evidence of the incidence of diseases of the large bowel in different communities consuming diets which differed in their fiber contents, among other things, and in communities where the diets had undergone a change in fiber content with time.

Since this paper a number of authors have suggested that a low fiber intake may be a contributory factor in the etiology of many of the diseases common to Western civilized man. Critical examination of the actual evidence available suggests that some of these associations are not very close² but does indicate that the role of these dietary components warrants more thorough investigation.

One of the early difficulties in the interpretation of the dietary data was one of terminology combined with the use of crude fiber values. These are unreliable estimates of the indigestible plant material in the diet³ and considerably underestimate the structural plant polysaccharides which are not digested by the secretions of the human digestive tract.⁴ The term dietary fiber⁵ was suggested as a term to cover all

the indigestible structural plant matter in the diet.

The precise way in which the dietary fiber exerts its protective effects are uncertain. Unquestionably it provides bulk in the large intestine and leads to the production of a larger stool volume which is usually moister than on a low dietary fiber diet.

A large number of clinical trials undertaken with the intention of studying the role of dietary fiber have used wheat bran as the source of dietary fiber and in some respects at least, conflicting results have been obtained.

In a recent paper⁶ evidence has been produced that the physical state and particle size of the bran used have a profound influence on its colonic function. This paper also points to some of the deficiencies of current thinking in this area.

The study involved two groups of patients before and after consuming 10 g of bran twice daily for four weeks. One group took a coarse preparation with particles greater than 1 mm in diameter, the other a fine bran obtained by sieving a wholemeal flour. This bran passed entirely through a 1 mm sieve and also contained a higher proportion of starchy endosperm.

The nine subjects having the coarse bran all had proven diverticular disease and of the five having the fine bran three had diverticular disease and two had a non-specific constipation with occasional abdominal pain. Colonic mobility was measured using open-ended tubes and transit time using radio-opaque pellets in coarse bran group, and with a radio-isotope capsule in the fine bran group. The measurements were made before treatment and after four weeks on the appropriate

bran. Four patients treated with the fine bran initially were also studied after four weeks treatment with the coarse bran.

The basal motility index was lowered in the coarse bran group but not significantly, although when measured after food there was a significant lowering. The transit time was also significantly reduced. In the fine bran patients all the motility indices were reduced and the transit times fell but these changes were not significant. Comparative statistical analysis indicated that there was a significant difference between the effects of the two brans on motility after food and after treatment with neostigmine. In the patients who were tested on the coarse bran after the initial treatment with fine bran a similar type of effect was observed.

In addition to particle size the two bran preparations differed in other characteristics. Their moisture and protein contents were quite similar but the coarse bran contained 15.1 percent acid-detergent fiber compared with 9.65 percent in the fine bran and the lignin contents were 4.1 and 2.63 percent respectively. The brans also differed in their capacity to absorb water by a factor of nearly 3 to 1 and had slightly different cation exchange properties. When the coarse bran was milled to pass a 1 mm sieve the water-binding capacity of the coarse bran was reduced from 6.15 to 3.54 g per gram and that of the fine bran was reduced on remilling from 2.63 to 2.16 g per gram.

The authors, in their examination of these results, express some surprise at the difference in effect of the two brans on bowel function. One of the known beneficial effects of bran is probably due to its water-holding properties which are believed to be responsible for the soft, bulky, easily passed stools. These authors suggest that the water-binding properties of the dietary fiber components in the bran appear to depend on the physical state and size of the plant structural material in the bran. The in vitro measurements show very clearly that the two brans possessed different water-binding properties and that milling had a profound effect in reducing this water-

binding even in the fine bran. They conclude that the particle size of the bran preparations may be more important in its effects on bowel function than the actual amount of dietary fiber it provides.

A more detailed examination of this paper is, however, of interest because it raises some important points regarding studies of dietary fiber.

The groups of patients which were compared were not matched very closely, which may be inevitable in such clinical studies, and the variance in the transit times observed in the two groups was very different.

The amounts of acid detergent fiber fed to the patients were also quite different. The patients on the coarse bran received over 3.0 g per day compared with 1.9 g per day in the fine bran group.

Bran contains a considerable amount of hemicelluloses which are not measured in the acid-detergent-fiber procedure, so these values underestimate total dietary fiber intakes although probably proportionately in the two groups. It is therefore difficult to ascribe the differences in colonic behavior to particle size alone as the patients receiving coarse bran had nearly 50 percent more dietary fiber.

The efficacy of the structural plant polysaccharides in binding water in the large intestine depends on a number of factors of which the physical structure of the material is obviously one; another factor which must be equally important is the resistance of the polysaccharide to bacterial degradation. This is an area where there is a great deal of work to be done. It is known that in the ruminant³ the physical nature and pretreatment of the structural materials influence their degradation by the rumen microflora. Milling may increase the susceptibility of the plant cell wall material by exposing more surfaces to bacterial action and this may in itself reduce the capacity of the polysaccharides to bind water in the large intestine. The in vitro characterization of the water-binding properties of foods may well be correlated with water-binding in the large intestine but this has not yet been demonstrated.

An understanding of the true role of dietary fiber in human nutrition and disease depends on the integration of knowledge from a number of disciplines and it is important in clinical studies of the kind made by W. O. Kirwan et al.⁶ that very careful control of the source, and chemical and physical nature of the dietary fiber fed is exercised. This must also be accompanied by the development of a more detailed understanding of the metabolism of these complex plant polymers in the large intestine itself. □

1. N. S. Painter and D. P. Burkitt: Diverticular Disease of the Colon: A Deficiency Disease of

Western Civilization. *Brit. Med. J.* 2: 450-454, 1971

2. J. H. Cummings: Dietary Fibre. *Gut* 14: 69-81, 1973
3. P. J. Van Soest and R. W. McQueen: The Chemistry and Estimation of Fiber. *Proc. Nutrition Soc.* 32: 123-130, 1973
4. D. A. T. Southgate: Fiber and the Other Unavailable Carbohydrates and Their Effects on the Energy Value of the Diet. *Proc. Nutrition Soc.* 32: 131-136, 1973
5. H. C. Trowell: Dietary Fibre and Coronary Heart Disease. *Rev. Eur. Etud. Clin. Biol.* 17: 345-348, 1972
6. W. O. Kirwan, A. N. Smith, A. A. McConnell, W. D. Mitchell, and M. A. Eastwood: Action of Different Bran Preparations on Colonic Function. *Brit. Med. J.* 4: 187-189, 1974

EFFECTS OF DIET ON BILIARY LIPID SECRETION AND BILE COMPOSITION

Changes in the nature of the dietary fat have important effects upon the composition and lithogenicity of bile.

Key Words: cholesterol, bile, phospholipids, gallstones, dietary fat

Gallstones in the United States and in Europe are predominantly cholesterol. A prerequisite for cholesterol stone formation is for the bile to contain a higher concentration of cholesterol than the combined solubilizing effects of bile salts and lecithin. This supersaturated bile is commonly referred to as lithogenic bile. Factors which modify the relative rate of excretion of any of these three components would either promote cholesterol stone formation or prevent such formation.

L. DenBesten and her co-workers¹ measured the composition of bile in 14 subjects undergoing dietary manipulation. Ten were normal men, three were patients studied after a cholecystectomy, and one had type II hypercholesterolemia. Bile samples were obtained by duodenal intubation except for the patients after cholecystectomy who had an occlusive tube in the common bile duct permitting either

sampling or passage of bile into the duodenum.

The diet was cholesterol free eucaloric formula made up with casein as 15 percent total calories, carbohydrate 45 percent (20 percent cornstarch, 26 percent dextramaltose, 44 percent sucrose), and fat 40 percent. Vitamin and minerals were added. Cholesterol was substituted for fat and protein and added in the form of egg yolk to the level of 750 mg daily. Each diet was fed in random order for 21 days and then single samples of bile taken for analysis. Each subject received at least one cholesterol free and one cholesterol period.

In the normal subjects, cholesterol feeding was associated with a significant rise in the percent of total moles as cholesterol from 8.05 ± 0.62 to 10.88 ± 0.95 (mean and SE) and of phospholipid from 24.16 ± 1.78 to 30.35 ± 2.62 whereas bile acid decreased from 68.32 ± 1.92 to 58.7 ± 3.39 . The individual bile acids making up the total were measured but

showed no significant differences. Similar changes were found in several studies on the hypercholesterolemic patient. In the patients with T tubes permitting total bile collection following cholecystectomy the increased cholesterol output was 34.3 percent or 222 mg cholesterol. The increase in phospholipids was 29.6 percent and total bile acid secretion rose in these patients by 20.6 percent. The total effect of 750 mg of additional cholesterol in the diet was a significant elevation in both absolute and mole percent of cholesterol in the bile in all subjects. Four of the ten normal subjects converted bile from nonlithogenic to lithogenic by the single sample obtained. There was wide variation from individual to individual in the magnitude of changes produced by the increased cholesterol in the diet.

A closely related study was conducted by R.N. Redinger and his co-workers² in the rhesus monkey. The biliary flow was diverted externally through a stream splitter such that a constant 5 percent of all flow could be saved for analysis and the majority constantly returned to the duodenum. Monkeys were maintained on a chow diet or diets in which 30 percent of the calories were administered as safflower oil, triolein, or tricaprylin or an isocaloric fat-free diet. Diets were fed for two to three weeks to obtain steady state conditions before bile samples were obtained. Bile was analyzed as 5 percent of the total bile produced in 24 hours. Safflower oil and triolein increased bile flow over control significantly (from approximately 200 on control to 250 ml per 24 hours) and both diets increased bile salt secretion approximately 50 percent. Lecithin excretion increased with both diets; cholesterol secretion was variable on the safflower oil but increased from 0.160 to 0.284 moles per 24 hours on triolein. Tricaprylin feeding was not associated with a significant change in bile flow, but resulted in a significant decrease in bile salt secretion. Cholesterol secretion was significantly decreased by tricaprylin in the diet. The fat-free diet decreased bile salt secretion significantly but did not significantly alter bile flow or

cholesterol secretion. Fasting for periods up to six days produced a progressive fall in bile flow and bile salt secretion rates with a less striking but similar change in phospholipid and cholesterol secretion.

These various diets did not produce lithogenic bile but both tricaprylin and fat-free diet resulted in a decrease in the relative proportion of cholesterol in the bile. Fasting produced a relative decrease in bile salt but at no time was this sufficient to make the bile lithogenic. The model readily permitted the measurement of the bile salt synthesis rate. Long chain triglycerides increased the bile salt synthesis over the controls, over tricaprylin, over the fat-free diet, and over fasting. Compared with the control diet bile salt synthesis was decreased in the tricaprylin diet, the fat-free diet, and during fasting.

These two studies are remarkably similar between man and the rhesus monkey. Clearly dietary lipid composition influences the total composition of bile. In general a high lipid diet (of chain length greater than C-8) increases cholesterol, phospholipid, and bile salt excretion and a low lipid diet diminishes all components. Cholesterol in the diet is reflected similarly by all components of bile increasing. These components, however, do not change proportionately and high cholesterol diets in man are associated with a greater frequency of lithogenic bile.

The importance of dietary lipid, yet the difficulty in establishing how important these changes would be over many years, is beautifully reflected in an autopsy follow-up of the diet study of R. A. Sturdevant and his co-workers.³ Several thousand males were followed carefully during up to five years of ingesting a normal hospital diet or predominantly a cholesterol-lowering diet consisting of polyunsaturated fat and low cholesterol intake. A careful examination of the 72 patients who came to autopsy from the cholesterol-lowering diet indicated 34 percent had cholesterol gallstones whereas only 14 percent of the 89 patients autopsied from the control diet had them. Furthermore, there was a highly positive correlation between the number of

meals taken of the cholesterol-lowering diet and the frequency of cholesterol gallstones. Thus all patients ingesting more than 250 meals had increased gallstones, with greater than 1000 and greater than 2500 meals each providing a further increment in the probability of gallstones. These patients had lower serum cholesterol levels than did the control group indicating that the diets were highly effective.

This study confirms the effect of diet on the composition of bile and the propensity to form gallstones. The rhesus monkey study does not give a clear prediction as to this occurrence suggesting that chronic studies in man and animal may be necessary. It is clear that the diet fed by Sturde-

vant and co-workers created lithogenic bile and is an additional adverse effect to be held in concern. □

-
1. L. DenBesten, W. C. Connor, and S. Bell: The Effect of Dietary Cholesterol on the Composition of Human Bile. *Surg.* 73: 266-273, 1973
 2. R. N. Redinger, A. H. Hermann, and D. M. Small: Effects of Diet and Fasting on Biliary Lipid Secretion and Relative Composition and Bile Salt Metabolism in the Rhesus Monkey. *Gastroenterology* 64: 610-621, 1973
 3. R. A. Sturdevant, M. L. Pearce, and S. Dayton: Increased Prevalence of Cholelithiasis in Man Ingesting a Serum-Cholesterol-Lowering Diet. *New Engl. J. Med.* 288: 24-27, 1973

WATER AND ELECTROLYTES IN MALNUTRITION

Dermal and urinary losses of electrolytes are important therapeutic considerations in the rehabilitation of the malnourished child. A simple formula based on the use of cow's milk is suggested for the treatment of these children.

Key Words: potassium, electrolyte balance

There is already considerable literature on electrolyte metabolism in infantile malnutrition.¹ The major elements which have been considered are potassium, sodium, and magnesium, although recently the effects of trace element deficiencies are also being studied. Most attention has been paid to potassium, and the methods used to study potassium metabolism have included balance studies, ^{42}K , and whole body counting of ^{40}K . Since muscle is the major store of body potassium, muscle biopsies have also been used to give a measure of the distribution of the changes in body potassium. As a result of these various approaches, it has been established that in the severely malnourished child body potassium is reduced but this need not be viewed as a true deficiency of potassium since the body's capacity to retain and store potassium is also reduced.²

It has been shown by B. L. Nichols and his group³ that if malnourished children

are maintained on a low protein diet for a few days after admission, potassium retention is small, and muscle potassium does not change. As the protein and energy content of the diet are increased, however, the potassium retention also increases and muscle potassium rises to normal levels. More recently, the same workers concentrated on balance measurements made during recovery, the route of loss of these electrolytes, and have tried to devise a simple formula which can be used in the rehabilitation of these children.⁴

They have studied malnourished edematous children during the three phases of their hospitalization. In an initial test period, the diet provided 0.7 g of protein, and 80 calories per kilogram per day. This period they designated the "admission period", and it lasted approximately ten days. Thereafter, in the "therapeutic period", the diet was increased to 3 g of protein and 120 calories per kilogram per day. Throughout both periods KCl was

given as a supplement of 4 mEq per kilogram per day. Finally the children were studied on recovery. The major methods used were electrolyte balances and measurement of total body potassium by ^{42}K dilution; special attention was also paid to insensible water loss and the potassium content of the dermal losses.

In the early admission period, the children were in negative sodium and nitrogen balance, but in positive potassium balance. There was a minor increase in total body potassium. During the therapeutic period, there was avid sodium and nitrogen retention and potassium retention continued. Total body potassium continued to rise. Insensible water loss increased markedly from admission to the therapeutic and recovery periods. Because the children invariably had diarrhea, the fecal loss in the admission period was considerably higher than in the other two periods. Predictably there was a significant correlation between the fecal mass and fecal potassium loss. The urine volume rose from admission to the therapeutic period, but fell to its lowest level at full recovery. This work documents for the first time that there can be significant dermal losses of potassium in malnourished and recovering children. It has been recorded previously that in children recovering from malnutrition there was considerable postprandial sweating. Earlier workers had regarded this as a characteristic of the nutritional recovery syndrome.

This study also points out that since renal concentrating ability is impaired, the urine volume becomes a function of solute load with consequent electrolyte losses. There is an impressive correlation between protein or nitrogen intake and urine volume. Another important point is made regarding brain potassium. It had been shown that in spite of potassium retention in the admission period, there is no change in muscle or liver potassium, which is in consonance with J. S. Garrow's earlier observation.⁵ He postulated that the potassium retention of this early period is probably a function of increasing brain potassium.

The therapeutic implications discussed in this study are perhaps its most important aspect. In the admission period, there is a danger of salt and water overload because of renal functional impairment, as well as the danger of potassium depletion during gastrointestinal and renal losses. It is pointed out that if cow's milk were used alone, in order to achieve 100 calories per kilogram per day, then 150 ml per kilogram would have to be given, and this would provide 5.4 g of protein per kilogram. At this level of solute load, there would be a tremendous solute diuresis perhaps with dehydration. If a formula similar to human milk were used, this would provide adequate protein, but would give far more sodium than the infant could handle, and the potassium would be grossly inadequate. They recommend a modified milk formula consisting of a dilution of two parts of whole cow's milk with one part of a 15 percent dextrimaltose solution containing 1.5 percent KCl. This mixture would provide adequate protein, calories, potassium, and sodium at a level which would not precipitate cardiac failure. Another important point is that the full therapeutic diet should be introduced gradually in a step-wise manner.

A major virtue of this study is that it suggests a simple therapeutic approach based on data obtained by careful balance studies and electrolyte measurements. A few minor modifications could be suggested, such as the addition of magnesium to the formula. It has been shown conclusively that malnourished children are magnesium depleted,⁶ and as a consequence may have serious cardiac problems. It must also be borne in mind that a generalized recommendation, such as the one made in this study, will be suitable for the majority of children. The very ill, acidotic malnourished infant with severe gastroenteritis who presents with edema as well as hyponatremia and potassium depletion will require the careful monitoring which can only be done in special units. The point is always made, however, that if any serious attempt is made to rehabilitate malnourished children, there has to be a simple

formula which can be used in any field station by any group of workers without sophisticated equipment or a great deal of scientific training. Perhaps each of the countries in which malnutrition is still endemic would be well advised to prepare some sort of simple manual which can be used empirically in the treatment of the majority of these ill children. This of course must go hand in hand with the obvious attempts at prevention and education, which need not nor cannot be reviewed here. □

1. J. S. Garrow, R. Smith, and E. E. Ward in *Electrolyte Metabolism in Severe Infantile Malnutrition*. Pergamon Press, Oxford and New York, 1968

2. G. A. O. Alleyne, D. J. Millward, and G. S. Scullard: Total Body Potassium, Muscle Electrolytes, and Glycogen in Malnourished Children. *J. Pediat.* 76: 75-81, 1970
3. B. L. Nichols, J. Alvarado, C. F. Hazlewood, and F. Viteri: Clinical Significance of Muscle Potassium Depletion in Protein-Calorie Malnutrition. *J. Pediat.* 80: 319-330, 1972
4. B. L. Nichols, J. Alvarado, J. Rodriguez, C. F. Hazlewood and F. Viteri: Therapeutic Implications of Electrolyte, Water, and Nitrogen Losses during Recovery from Protein-Calorie Malnutrition. *J. Pediat.* 84: 759-768, 1974
5. J. S. Garrow: Loss of Brain Potassium in Kwashiorkor. *Lancet* II: 643-645, 1967
6. J. L. Caddell and D. R. Goddard: Studies in Protein-Calorie Malnutrition. I. Chemical Evidence for Magnesium Deficiency. *New Engl. J. Med.* 276: 533-535, 1967

GENU VALGUM DUE TO FLUORIDE TOXICITY

Fluoride toxicity under certain circumstances can lead to osteoporosis and knock knee (genu valgum) besides the usual pathology of sclerosis of the bone and calcification of the soft tissues. In certain parts of south India where fluorosis is endemic, genu valgum has appeared only in recent years and has become a major public health problem. Etiology of this modified picture of fluorosis is unknown.

Key Words: fluorosis, sclerosis, osteoporosis

The classical features of fluoride toxicity are dental mottling and skeletal manifestations such as osteosclerosis and calcification of ligaments, membranes, and tendinous insertions. These lead to crippling deformities such as kyphosis, stiffness of the spine, reduced mobility of the bony cage, and bony exostoses.

Fluorosis has been known to be endemic in certain parts of India such as Punjab and Uttar Pradesh in the north and Andhra Pradesh and Tamil Nadu in the south. According to earlier reports, crippling skeletal changes due to sclerosis appear primarily in adults after three to four decades of residence in the endemic area.

The problem of fluorosis in south India seems to have acquired a new dimension in recent years. K.A.V.R. Krishnamachari and Kamala Krishnaswamy recently reported

widespread prevalence of genu valgum (knock knee) in areas where fluorosis has been endemic.¹⁻⁴ Apparently this pathology is of recent origin because it was not reported earlier, and it is now only seen in subjects between the ages of 10 to 25 years. The older population in the same area suffers from dental fluorosis and sclerotic skeletal changes, but not genu valgum.

Unlike the other clinical features of fluorosis, this syndrome is seen only among the poor whose staple is sorghum (in some cases rice) and whose diet is lacking in other protective foods. The patients suffer from gross skeletal deformities of the lower limbs, severe physical, economic, and social handicaps, and are psychologically disturbed.

Radiological examination revealed osteosclerosis and "bamboo spine" due to

calcification of the spinal ligaments. In all the subjects examined there was sclerosis of the humerus, scapulae ribs, radius, ulna, and pelvic bones together with calcification of muscular attachments. By contrast, the most striking radiological feature was severe osteoporosis of the lower end of the femur and the upper ends of the tibia and fibula and rarefaction of the metacarpal bones. Serum calcium/phosphorus and alkaline phosphatase were within normal levels. Clinical evidence of rickets was absent in children under five years of age, indicating that primary dietary deficiency of vitamin D is probably not a causative factor.

An epidemiological survey of the affected villages revealed a prevalence rate of 1 to 17 percent with an average of 3.6 percent. Males were predominantly affected with the ratio of afflicted males to females being 10:1. In most cases the deformity of the lower limbs was reported to have set in at the age of six to seven years and slowly increased in severity by the age of 15 years. The onset was insidious and the progression slow.

Presently, the etiology of this rare syndrome of fluoride toxicity is obscure. Analysis of the food grains grown in the endemic area revealed a higher concentration of molybdenum.³ In view of the molybdenum-copper interrelationship and the role of copper in collagen synthesis, this observation needs further investigation. Dietary surveys indicate inadequate calcium intake (300 mg per day) in this community. This is markedly lower than the calcium intake in endemic areas of northern India (900 to 1200 mg per day) where this syndrome has hitherto not been seen.⁵

A search for a recent ecological change which might have modified fluoride toxicity, shows a correlation between the prevalence of genu valgum and the recent construction of dams in south India. Such dams may alter the soil mechanics and change the subsoil mineral composition.

Apart from these recent reports from India, there is only one other report of genu valgum related to fluoride toxicity. In 1962 W.P.U. Jackson⁶ described osteoporosis and knock knee in an isolated community in a remote area of south Africa where fluorosis was endemic. The magnitude of the problem was limited both in severity and extent. The disease was primarily seen in the black population, probably due to poorer economic status. The calcium content of the water was adequate. Since the village was vacated, the authors could not study the disease further. Interestingly, there is a dam (Rooiberg Dam) in the vicinity of this African village. □

-
1. K.A.V.R. Krishnamachari and Kamala Krishnaswamy: Genu Valgum and Osteoporosis in an Area of Endemic Fluorosis. *Lancet* II: 877-879, 1973
 2. K.A.V.R. Krishnamachari and Kamala Krishnaswamy: An Epidemiological Study of the Syndrome of Genu Valgum among Residents of Endemic Areas for Fluorosis in Andhra Pradesh. *Ind. J. Med. Res.* 62: 1415-1423, 1974
 3. Studies on Fluorosis. Annual Report, National Institute of Nutrition, Hyderabad, India. Pp. 123-125, 1974
 4. K.A.V.R. Krishnamachari: Some New Aspects of Fluorosis in South India. A New Approach to Prevent the Problem. Proceedings of the Symposium on Fluorosis sponsored by the Indian Academy of Geoscience and others, Hyderabad, India, 1974
 5. S. S. Jolly, S. Prasad, and R. Sarma. Epidemiological Studies of Endemic Fluorosis in Punjab. Proceedings of the Symposium on Fluorosis sponsored by the Indian Academy of Geoscience and others, Hyderabad, India, 1974
 6. W. P. U. Jackson: Further Observations on the Kenhardt Bone Disease and Its Relation to Osteoporosis. *S. Afr. Med. J.* 36: 932-936, 1962

INTESTINAL BYPASS SURGERY FOR OBESITY

Jejunioileostomy is an effective way of treating intractable severe obesity and one that leads to marked subjective improvement. The hepatic lipid doubles in the postoperative period and the long term consequence of this is unknown.

Key Words: obesity, jejunio-ileal bypass, hepatic lipid, psychosocial associations of obesity

Previous reviews^{1,2} have dealt with some of the reports on the use of intestinal bypass surgery for the treatment of massive obesity and the problems associated with this approach. Although the jejunio-ileal shunt has gained in favor over the jejunocolic bypass originally employed, the reports of hepatic complications following this operation have been conflicting.³ R. T. Holzbach and his colleagues⁴ studied this question by performing histologic and chemical analyses of liver samples from patients undergoing surgical treatment for obesity. There were 15 female and eight male patients ranging from 18 to 53 years of age, whose obesity expressed as a percent excess of their ideal body weight was 80 to 230 percent. Prior to operation all were healthy although some had previously suffered from complications of obesity such as thrombophlebitis and pulmonary emboli. Dietary treatment had been attempted without success. All the patients underwent an end to end jejunio-ileal bypass with 25 cm of jejunum and ileum in continuity. During the operation a wedge section of the liver was obtained. Needle biopsies were also taken five to 24 months after the operation. A small portion of each biopsy sample was fixed for histological examination. The remainder was analyzed chemically for total lipid and triglyceride content. When possible total protein, phospholipids, and free and esterified cholesterol were also determined. Only three of the 23 patients had both histologic and chemical analyses of both the intraoperative and postoperative biopsy

samples, 11 had histologic examination only of both samples, and the remainder had histologic examination of the sample taken at operation but both types of analysis on the postoperative specimen. Histologically the specimens were graded as minimal if thought to contain less than 10 percent lipid, moderate for 10 to 50 percent, and marked if the fat content was greater than 50 percent, care being taken to minimize within and between observer bias.

The operation had a dramatic effect on body weight, reducing the degree of obesity from a mean of 137 percent to 42 percent. Chemical analysis of the liver biopsies showed that the mean triglyceride and total lipid concentration in 13 postoperative specimens were double the levels measured at operation (12 cases), being 222 and 177 mg per gram liver respectively after the operation and 111 and 80 mg per gram at operation. Both levels of total lipid were, however, greater than that of four non-obese subjects in whom the mean total hepatic lipid concentration was 41.8 ± 2.9 mg per gram. The postoperative rise in total lipid and triglyceride was not shared by the phospholipid, free cholesterol, and cholesterol esters. In the normal subjects triglycerides made up 27.5 percent of the total lipids, whereas in the obese patients the range was from 22.5 to 98.9 percent. An important finding came from the comparison of the triglyceride concentration measured chemically and a histological examination of the same specimen. Specimens with a similar mean triglyceride concentration were classified equally as mild or moderately fatty by morphology but better correlation was observed in those specimens which were thought to show marked

fat deposition. The poor correlation between the morphological and chemical characteristics of the tissue may come as no surprise to the uncommitted reader. It does emphasize however, as was the authors' intent, the limited reliance that can be placed on histological results expressed semi-quantitatively. No histologic relationship was found between the deterioration in hepatic lipid status and the interval between operation and the postoperative biopsy sample.

The authors claim, convincingly, to have shown a significant net hepatic fat accumulation as a result of the jejuno-ileal shunt operation and point out that the failure of other workers to demonstrate the same findings probably stems from the use of histologic assessment without careful standardization. The mechanism of the postoperative lipid accumulation has not yet been defined but in one of the 23 patients there was a fall from marked fat infiltration seven months after operation to minimal infiltration at 20 months. The other patients will continue to be studied to see if a late improvement occurs in them also.

In contrast with the possibly undesirable effects of bypass surgery on hepatic lipid, C. Solow and his colleagues⁵ report favorably on the psychosocial results of this form of treatment. They studied 32 consecutive obese patients treated by an end to side jejunoileostomy. One patient died on the fifth postoperative day from a pulmonary embolus and in two others sprue and ulcerative colitis developed which necessitated reanastomosis. The other 29 patients had a semi-structured psychiatric interview between one day and seven months before their operation, were followed in the hospital after the operation, and at six monthly intervals following discharge for a period of 26 to 46 months. A variety of psychiatric questionnaires and tests were performed during these interviews. The patients were 39 to 160 kg overweight, most having become so before the age of 16 years. Their eating patterns varied widely and all had tried different forms of

dietary treatment, without lasting success. Motivation for the operation was mixed in most cases but 11 sought surgery primarily because of somatic concern whereas the remainder were more upset by the psychosocial aspects of their obesity. Preoperative psychiatric assessment found 17 patients to be reasonably well-adjusted, but 12 to be abnormal. Psychiatric diagnoses comprised five cases of neurosis, four of personality disorder, and three with schizophrenia. Features common to most of the group were: loss of self-esteem, restriction of physical and social activity, severe distortion of body image, and strong fear of rejection with associated compulsions to please others and difficulty in self-assertion.

In three subjects the weight loss after surgery was unsatisfactory (4.5 to 15 kg), in some the weight loss fulfilled preoperative expectations but in the majority the result, although less than that desired, was regarded as substantial and gratifying. Weight loss occurred mainly in the first six months and weight stabilized up to 18 months after surgery. In 27 patients diarrhea was troublesome during the period of weight loss, became less when their weight had stabilized, but seven remained persistently bothered by flatulence, abdominal distention, and diarrhea. Once over the operation 21 reported an improvement in energy and stamina and a distinct improvement in their previous physical disabilities.

The investigators formed the general impression in their follow-up interviews of a surprising degree of improvement in psychosocial functioning. Loss of self-consciousness coupled with that in weight led to an increase in activity e.g. house-bound patients began to go out shopping. The psychological benefits were cumulative; as patients went out more they developed more social contacts and their self-esteem improved. Body image, considered in emotional terms, changed from self-loathing to realistic acceptance in 23 patients even when an appreciable degree of overweight persisted.

The most dramatic sensation in 12 patients was that the operation had broken a vicious cycle and given them a feeling of escape from entrapment or unrelieved failure. The authors emphasize how much of psychological disturbance associated with obesity may stem from the obesity and its chronicity. The impact of surgical treatment was profound because it gave the patients their first experience of success over their condition. It remains to be seen, as with the physical sequelae of this approach to the treatment of obesity, whether the short term benefits are consolidated over a longer period. If they are, a surgical approach may offer relief to many unhappy fat people. □

1. Current Status of Jejunio-Ileal Bypass for Obesity. *Nutrition Reviews* 32: 333-336, 1974
2. Obesity, Jejunio-Ileal Bypass and Death. *Nutrition Reviews* 33: 38-40, 1975
3. H. W. Scott Jr., H. H. Sandstead, A. B. Brill, H. Burko, and R. K. Younger: Experience with a New Technic of Intestinal Bypass in the Treatment of Morbid Obesity. *Ann. Surg.* 174: 560-571, 1971
4. R. T. Holzbach, R. G. Wieland, C. S. Lieber, L. M. DeCarli, K. R. Koepke, and S. G. Green: Hepatic Lipid in Morbid Obesity. Assessment at and Subsequent to Jejunioileal Bypass. *New Engl. J. Med.* 290: 296-299, 1974
5. C. Solow, P. M. Silberfarb, and K. Swift: Psychosocial Effects of Intestinal Bypass Surgery for Severe Obesity. *New Engl. J. Med.* 290: 300-304, 1974

THE LANCET.

A Journal of British and Foreign Medicine, Surgery, Obstetrics, Physiology,
Chemistry, Pharmacology, Public Health, and News.

Volume II: 1616-1620, 1902

THE INCIDENCE OF ALKAPTONURIA: A STUDY IN CHEMICAL INDI- VIDUALITY.

By ARCHIBALD E. GARROD, M.A., M.D. OXON.,
F.R.C.P. LOND.

*Physician to the Hospital for Sick Children, Great Ormond-
Street; Demonstrator of Chemical Pathology at
St. Bartholomew's Hospital.*

All the more recent work on alkaptonuria has tended to show that the constant feature of that condition is the excretion of homogentisic acid, to the presence of which substance the special properties of alkapton urine, the darkening with alkalis and on exposure to air, the power of staining fabrics deeply, and that of reducing metallic salts, are alike due. In every case which has been fully investigated since Wolkow and Baumann¹ first isolated and described this acid its presence has been demonstrated and re-examination of the material from some of the earlier cases also has led to its detection.

• • •

Lastly, Professor Osler supplies the very interesting information that of two sons of the alkaptonuric man previously described by Dr. Futcher¹³ one is alkaptonuric. This is the first known instance of direct transmission of the peculiarity. The parents of the father, who has an alkaptonuric brother whose case was recorded by Marshall,¹⁴ were not blood relations. The above particulars are embodied with those of the congenital British cases previously recorded in the following tabular epitome (Table III.).

TABLE III.—*Showing the large Proportion of Alkaptonurics who are the Offspring of Marriage of First Cousins.*

A.			
Families the offspring of marriages of first cousins.			
No.	Total number of family.	Number of known alkaptonuric members.	Observers.
1	14	4	Pavy.
2	4	3	R. Kirk.
3	5	2	A. E. Garrod.
4	1	1	Erich Meyer.
5	8	1	H. Ogden.
6	4	1	Hammarsten.
Total. . .	36	12	—

The question of the liability of children of consanguineous marriages to exhibit certain abnormalities or to develop certain diseases has been much discussed, but seldom in a strictly scientific spirit. Those who have written on the subject have too often aimed at demonstrating the deleterious results of such unions on the one hand, or their harmlessness on the other, questions which do not here concern us at all. There is no reason to suppose that mere consanguinity of parents can originate such a condition as alkaptonuria in their offspring, and we must rather seek an explanation in some peculiarity of the parents, which may remain latent for generations, but which has the best chance of asserting itself in the offspring of the union of two members of a family in which it is transmitted. This applies equally to other examples of that peculiar form of heredity which has long been a puzzle to investigators of such subjects, which results in the appearance in several collateral members of a family of a peculiarity which has not been manifested at least in recent preceding generations.

It has recently been pointed out by Bateson¹⁶ that the law of heredity discovered by Mendel offers a reasonable account of such phenomena. It asserts that as regards two mutually exclusive characters, one of which tends to be dominant and the other recessive, cross-bred organisms will produce germinal cells (gametes) each of which, as regards the characters in question, conforms to one or other of the pure ancestral types and is therefore incapable of transmitting the opposite character. When a recessive gamete meets one of the dominant type the resulting organism (the zygote) will usually exhibit the dominant character, whereas when two recessive gametes meet the recessive character will necessarily be manifested in the zygote. In the case of a rare recessive characteristic we may easily imagine that many generations may pass before the union of two recessive gametes takes place. The application of this to the case in question is further pointed out by Bateson, who, commenting upon the above observations on the incidence of alkaptonuria, writes as follows:¹⁷ "Now there may be other accounts possible, but we note that the mating of first cousins gives exactly the conditions most likely to enable a rare, and usually recessive, character to show itself. If the bearer of such a gamete mate with individuals not bearing it the character will hardly ever be seen; but first cousins will frequently be the bearers of similar gametes, which may in such unions meet each other and thus lead to the manifestation of the peculiar recessive character in the zygote." Such an explanation removes the question altogether out of the range of prejudice, for if it be the true account of the matter it is not the mating of first cousins in general but of those who come of particular stocks that tends to induce the development of alkaptonuria in the offspring. For example, if a man inherit the tendency on his father's side his union with one of his maternal

first cousins will be no more liable to result in alkaptonuric offspring than his marriage with one who is in no way related to him by blood. On the other hand, if members of two families who both inherit the strain should intermarry the liability to alkaptonuria in the offspring will be as great as from the union of two members of either family, and it is only to be expected that the peculiarity will also manifest itself in the children of parents who are not related. Whether the Mendelian explanation be the true one or no there seems to be little room for doubt that the peculiarities of the incidence of alkaptonuria and of conditions which appear in a similar way are best explained by supposing that, leaving aside exceptional cases in which the character, usually recessive, assumes dominance, a peculiarity of the gametes of *both* parents is necessary for its production.

• • •

A third condition which suggests itself as being probably another chemical "sport" is cystinuria. Our knowledge of its incidence is far more incomplete and at first sight direct inheritance appears to play here a more prominent part. However, when more information is forthcoming it may turn out that it is controlled by similar laws.

• • •

If it be, indeed, the case that in alkaptonuria and the other conditions mentioned we are dealing with individualities of metabolism and not with the results of morbid processes the thought naturally presents itself that these are merely extreme examples of variations of chemical behaviour which are probably everywhere present in minor degrees and that just as no two individuals of a species are absolutely identical in bodily structure neither are their chemical processes carried out on exactly the same lines. Such minor chemical differences will obviously be far more subtle than those of form, for whereas the latter are evident to any careful observer the former will only be revealed by elaborate chemical methods, including painstaking comparisons of the intake and output of the organism. This view that there is no rigid uniformity of chemical processes in the individual members of a species, probable as it is *a priori*, may also be arrived at by a wholly different line of argument. There can be no question that between the families, genera and species both of the animal and vegetable kingdoms, differences exist both of chemical composition and of metabolic processes.

• • •

If it be a correct inference from the available facts that the individuals of a species do not conform to an absolutely rigid standard of metabolism, but differ slightly in their chemistry as they do in their structure, it is no more surprising that they should occasionally exhibit conspicuous deviations from the specific type of metabolism than that we should meet with such wide departures from the structural uniformity of the species as the presence of supernumerary digits or transposition of the viscera.

CALCIUM TRANSPORT IN THE ILEUM

The ileum responds to dietary calcium; high levels induce an increase in movement from tissue to gut lumen producing net secretion; low levels induce an increase in movement from lumen to blood producing net absorption.

Key Words: ileum, mucosa, serosa, calcium secretion, calcium absorption

Substantial amounts of calcium are secreted into the gut. Although most studies have emphasized the control of calcium absorption from the gut, it is clear that the reverse process, calcium secretion, might represent a means by which the total body calcium is controlled.¹ To determine how this process works, the unidirectional movements of calcium in the ileum from lumen to blood and from blood to the lumen were studied in adult and growing rats fed high (HCD) and low (LCD) calcium diets.²

A group of 85 rats weighing between 75 to 95 g were randomized by weight. One group was fed the LCD, 0.02 percent calcium and 0.5 percent phosphorus; the other group was fed the HCD, 2.0 percent calcium and 0.5 percent phosphorus by weight. The movement of calcium was studied in vitro using an Ussing type apparatus and radioactive calcium.

The animals were raised on the diets for 15 weeks. After seven weeks, the LCD group demonstrated a significant reduction in growth rate remaining below that of the HCD group for the remainder of the study. At three, five, and 15 weeks the HCD group showed consistent secretion of calcium into the lumen of the ileum. It was concluded that secretion was active since the calcium flux could not be accounted for by passive forces. The secretion appeared to be a part of a gradual increase (over the three to 15 week period) of the movement of calcium from the serosal bathing media to the mucosal bathing media. Although a gradual increase in the

movement from the mucosa to the serosa occurred, the increase in serosal to mucosal movement was higher, always resulting in a net secretion of calcium. On the other hand, the LCD group showed essentially similar rates in both unidirectional movements of calcium after three and 15 weeks. There was no absorption or secretion. At five weeks, however, absorption of calcium was demonstrated. Blood concentrations of phosphorus were similar in both groups; magnesium, however, was much higher in the LCD group. Blood calcium tended to be lower in the LCD group. Blood calcium increased in both groups of rats during the 15 week period of study. The increase was negatively correlated with the weekly change in weight ($r = -0.97$).

Duodenal transport of calcium was also studied in the HCD animals. It was found that the duodenum did not behave like the ileum. Absorption of calcium occurred despite the HCD diet. The movement of calcium from the serosa to the mucosal solution was three-fold less in the duodenum than in the ileum. Since the resistance, measured electrically across the full thickness of the gut, was much less in the ileum, it was concluded that a larger diffusion pathway was present in the ileum. This conclusion did not appear to support the authors' previous interpretation of the data, however, in which serosal to mucosal flux was considered active.

Movement of calcium from mucosa to serosa and from serosa to mucosa increased as the concentration of calcium in the serosal or mucosal bathing solutions increased. The movement from serosal to mucosal solutions, however, appeared much more sensitive to ionic calcium; in-

creasing 100-fold versus a 30-fold increase in the opposite direction when bathing solution concentrations were increased from 0.125 to 10 mM. This differential effect of calcium in the bathing solution resulted in secretion of calcium in all experiments except at the 0.125 mM concentration in which absorption was noted.

Thus, higher concentrations of media calcium favored the unidirectional movement of calcium from serosal to mucosa and produced net secretion. How this related to in vivo conditions is not clear. Probably the HCD diets result in absorption in the duodenum but this increases the calcium concentration in the blood which may stimulate an increase in ileal movement from blood to gut lumen. The net calcium balance would thus be related to the proportional changes in the two opposing processes.

The feeding of low or high calcium diets may, of course, influence a variety of factors or processes, such as the levels of parathyroid hormone, skeletal growth, calcium transport, protein, etc. The integration of these systems is not understood. The studies do show, however, that the level of dietary calcium or the level of body calcium may trigger either calcium secretion or calcium absorption in the ileum. This apparently provides an additional controlling mechanism to allow adaptation to different levels of dietary calcium. □

1. M. W. Walling and D. V. Kimberg: Active Secretion of Calcium by Adult Rat Ileum and Jejunum In Vitro. *Am. J. Physiol.* 225: 415-422, 1973
2. M. W. Walling and D. V. Kimberg: Calcium Absorption or Secretion by Rat Ileum In Vitro: Effects of Dietary Calcium Intake. *Am. J. Physiol.* 226: 1124-1130, 1974

EFFECT OF VITAMIN B₁₂ DEPRIVATION ON CoA INTERMEDIATES RELATED TO PROPIONATE METABOLISM

Propionyl CoA is carboxylated to methylmalonyl CoA, which forms succinyl CoA in a coenzyme B₁₂-dependent mutase reaction. However, the level of propionyl CoA increases more than methylmalonyl CoA in the liver of B₁₂-deficient rats, and an increase rather than decrease in succinyl CoA occurs. Malate increases while citrate remains normal.

Key Words: vitamin B₁₂, coenzyme A intermediates, propionate metabolism

A portion of the propionyl CoA, derived from oxidation of odd-numbered fatty acids and from catabolism of such compounds as isoleucine, can be converted to one isomer of methylmalonyl CoA in a reaction catalyzed by a biotin-dependent carboxylase.¹ After an enzyme-catalyzed racemization,^{2,3} most of the second isomer of methylmalonyl CoA is normally converted to succinyl CoA in a vitamin B₁₂ coenzyme-dependent mutase reaction.^{4,5} The presence of tissue deacylase⁶ allows a fraction of the CoA esters to be hydrolyzed, so that both propionic and methylmalonic acidurias occur in experimental

and clinical deprivation of B₁₂ in animals and man.⁷ Also, because of the block in methylmalonyl CoA mutase in B₁₂ deficiency, an increased fraction of propionyl CoA is channeled toward synthesis of odd-chain fatty acids,⁸ which accumulate in the myelin of peripheral nerves.⁹

Although the function of B₁₂ in the metabolism of methylmalonyl CoA had been established at the enzyme level and some of the major sequelae resulting from insufficient mutase activity recognized, the actual levels of methylmalonyl CoA and its precursor and product present in the tissues of a B₁₂-deficient animal had not been measured. E. P. Frenkel et al. have now determined the levels of such CoA inter-

mediates associated with propionate metabolism in the livers of normal and B₁₂-deficient rats.¹⁰

Rats were maintained on rat chow which contained 9 µg of B₁₂ per pound, or a vitamin B₁₂-deficient mix which was without added B₁₂ and contained 0.05 percent iodinated casein. A third group of animals was given the B₁₂-deficient diet but supplemented with B₁₂ by intramuscular injection of 100 µg of cyanocobalamin given three times a week for two weeks before sacrifice. Development of the B₁₂-deficient state in rats not given the vitamin required approximately eight months and was serially evaluated by the decrease in serum B₁₂ levels and increase in propionic and methylmalonic acidurias. The spleen and liver contents of vitamin B₁₂ were also markedly decreased in the B₁₂-deficient rats, and liver weights, as well as the ratio of the weight of this organ compared to total body weight, was increased. In all cases except liver and body weights, the findings with the B₁₂-supplemented animals were similar to the controls that had received the vitamin admixed with the diet.

To assess the levels of CoA intermediates in liver under conditions that would reflect in vivo content, animals were stunned by a blow on the head, and a portion of the liver was rapidly freeze-clamped in situ. The frozen tissue was transferred into liquid nitrogen and pulverized in a pre-chilled mortar. Aliquots of the supernatant solutions obtained by centrifuging perchlorate-treated homogenates were assayed for CoA derivatives with and without addition of standards. Propionyl CoA was determined in neutralized extracts by carboxylating with H¹⁴CO₃ and propionyl CoA carboxylase to determine ¹⁴C incorporated. Succinyl CoA was measured by releasing reduced CoA with succinate thiokinase and arsenate and spectrophotometrically determining the sulfhydryl group by reaction with 5,5'-dithiobis (2-nitrobenzoic acid). Methylmalonyl CoA was determined by coupling the assay for succinyl CoA with a preparation of methylmalonyl CoA mutase, which also contained

the racemase. Methylmalonyl CoA content was then calculated to be the amount above that determined for succinyl CoA alone. Acetyl CoA was determined with citrate synthase and oxalacetate by again spectrophotometrically measuring the release of reduced CoA by its color reaction with the thionitrobenzoate reagent. Citrate and malate were quantitated by spectrophotometric assays of pyridine nucleotides in systems using citrate lyase plus malate dehydrogenase and malic enzyme, respectively.

Frenkel et al.¹⁰ carefully delineated the specificities and characteristics of the assay procedures used. The investigators found that mean propionyl CoA levels were 17-fold greater in the livers of the B₁₂-deprived group than in those of controls that had received the vitamin either orally or by injection. Although methylmalonyl CoA was increased approximately 12-fold in B₁₂ deficiency, the finding that the level of this substrate of the coenzyme B₁₂-dependent mutase was less elevated than its precursor was somewhat surprising. Also, it was expected that the level of the product of the methylmalonyl CoA mutase reaction, succinyl CoA, would be reduced in the livers of B₁₂-deprived animals, yet the opposite was found. The concentration of succinyl CoA was determined to be nearly four-fold higher. This was shown not be an artifact of tissue handling, such as could conceivably occur if its stability were significantly greater than propionyl CoA or methylmalonyl CoA in the time interval necessary to freeze the liver in situ.

A reciprocal relationship was found between the liver vitamin B₁₂ values and those for the levels of propionyl and methylmalonyl CoA. Propionyl CoA increased markedly above the normal range (3 to 5 nmoles per gram) when B₁₂ was determined to be less than 40 ng per gram of liver, which is about 30 percent of the normal B₁₂ level. Methylmalonyl CoA exhibited a similar increase from normal levels of almost 5 nmoles per gram when the B₁₂ content fell below 30 ng per gram of liver.

Levels of acetyl CoA were found to be almost twice as great in B₁₂-deficient as compared to control animals. Moreover, the concentration of malate was determined to be approximately two-fold greater in liver extracts from B₁₂-deficient animals, while the citrate level was not significantly different than that found in the controls.

The interesting and somewhat surprising findings of Frenkel et al.¹⁰ that there are such increases in the CoA intermediates following and even preceding methylmalonyl CoA in the mutase step dependent upon B₁₂ cannot be explained at present. Some of the possible causes, however, have been suggested. Explanations for the higher than expected increase in propionyl CoA may include the known reversibility of the carboxylase reaction, which is influenced by the relative mitochondrial levels of ATP, ADP, inorganic phosphate, and bicarbonate, as well as methylmalonyl CoA.¹¹ Further, there may be a relatively inefficient deacylation of propionyl CoA where the concentration of L-carnitine is not sufficient to handle the increase in propionate utilization. It is known that methylmalonyl CoA disappears as rapidly in tissue homogenates from B₁₂-deficient as from normal rats.⁶ This may be related to a deacylation mechanism that is more efficient for methylmalonyl CoA than for propionyl CoA. A possible explanation for increased malate is that the high propionyl CoA, and maybe even succinyl CoA, inhibits citrate synthase, causing a decrease in Krebs cycle activity. This might even explain the increase in succinyl CoA, which would not be catabolized as efficiently but would continue to be formed from glutamate via alpha-ketoglutarate. Again, though, experimental evidence is lacking. □

1. Y. Kazirow, E. Leone, and S. Ochoa: Biotin and Propionyl Carboxylase. *Proc. Nat. Acad. Sci. USA* 46: 1319-1327, 1960

2. R. Mazumder, T. Sasakawa, Y. Kazirow, and S. Ochoa: Metabolism of Propionic Acid in Animal Tissues. IX. Methylmalonyl Coenzyme A Racemase. *J. Biol. Chem.* 237: 3065-3068, 1962
3. P. Overath, G. M. Kellerman, F. Lynen, H. P. Fritz, and H. J. Keller: On the Mechanism of the Rearrangement of Methylmalonyl - CoA into Succinyl - CoA. II. Experiments on the Mechanism of Action of Methylmalonyl - CoA Isomerase and Methylmalonyl - CoA Racemase. *Biochem. Z.* 335: 500-518, 1962
4. M. Flavin and S. Ochoa: Metabolism of Propionic Acid in Animal Tissues. I. Enzymatic Conversion of Propionate to Succinate. *J. Biol. Chem.* 229: 965-979, 1957
5. E. R. Stadtman, P. Overath, H. Eggerer, and F. Lynen: The Role of Biotin and Vitamin B₁₂ Coenzyme in Propionate Metabolism. *Biochem. Biophys. Res. Commun.* 2: 1-7, 1960
6. G. J. Cardinale, P. M. Dreyfus, P. Auld, and R. H. Abeles: Experimental Vitamin B₁₂ Deficiency: Its Effect on Tissue Vitamin B₁₂-Coenzyme Levels and on the Metabolism of Methylmalonyl CoA. *Arch. Biochem. Biophys.* 131: 92-99, 1969
7. E. V. Cox, D. Robertson-Smith, M. Small, and A. M. White: The Excretion of Propionate and Acetate in Vitamin B₁₂ Deficiency. *Clin. Sci.* 35: 123-134, 1968
8. E. P. Frenkel, R. L. Kitchens, and J. M. Johnston: The Effect of Vitamin B₁₂ Deprivation on the Enzymes of Fatty Acid Synthesis. *J. Biol. Chem.* 248: 7540-7546, 1973
9. E. P. Frenkel: Abnormal Fatty Acid Metabolism in Peripheral Nerves of Patients with Pernicious Anemia. *J. Clin. Invest.* 52: 1237-1245, 1973
10. E. P. Frenkel, R. L. Kitchens, L. B. Hersh, and R. Frenkel: Effect of Vitamin B₁₂ Deprivation on the in Vivo Levels of Coenzyme A Intermediates Associated with Propionate Metabolism. *J. Biol. Chem.* 249: 6984-6991, 1974
11. M. J. Weidemann and H. H. Krebs: Acceleration of Gluconeogenesis from Propionate by DL-Carnitine in the Rat Kidney Cortex. *Biochem. J.* 111: 69-81, 1969

THYROID FUNCTION IN EXPERIMENTAL AND CLINICAL UNDERNUTRITION

Thyroidal activity is depressed in both clinical and experimental undernutrition probably due to hyposecretion of hypothalamic thyroid-releasing hormone.

Key Words: thyroid function, thyroid-stimulating hormone, thyroxine, thyrotropin-releasing hormone, caloric deprivation, neonatal rat, infantile malnutrition

A recent review¹ commented that there was general agreement from measurement of plasma hormone levels and metabolic rates that thyroidal function is depressed in malnutrition. A point of interest which remains unresolved is whether thyroidal hormone secretion is depressed ab initio and so causes a low rate of oxygen consumption or whether there is depressed metabolism because of substrate lack which by a feedback mechanism causes inhibition of thyroidal function. The latter mechanism might act directly on the thyroid gland but could be manifest by the brain at adenohypophyseal or hypothalamic levels. The development of assays for thyrotropin (TSH) and thyrotropin-releasing hormone (TRH)² have made it possible to investigate these hypotheses directly. G. E. Shambaugh III and J. F. Wilber³ chose the neonatal rat as the model for their experiments. Newborn rats were made hypothyroid by the addition of propyl thiouracil (PTU) to their mothers' drinking water from day 19 of pregnancy onwards. Other animals were deprived of milk by being removed from the mother for 16 out of each 24 hours. Control animals were allowed to feed continuously.

The effects of these treatments were assessed by measurement of the total body weight of the pups, the weight of both kidneys, and the weight of the gastrocnemius-plantaris muscle group. Prenatal exposure to PTU for two days had no effect on these three weights. The pups made hypothyroid by suckling from PTU

treated mothers also did not show a significant alteration in weight at four or eight days but were approximately 25 percent lighter in all three measurements by 16 days. The nutritionally deprived animals were more severely affected, showing significant reductions in all three weights from the fourth day, and by the sixteenth postnatal day being between 25 to 33 percent the weight of the control animals. The authors did not attempt to document the growth retardation in cellular terms by either morphometric or chemical means. It would have been of interest to know if the addition of an antithyroid drug to the maternal drinking water had the same type of action as caloric deprivation. Also unresolved are questions such as whether a PTU treated mother has as much milk as a control animal or if her offspring suckle as vigorously as normal pups.

The effectiveness of the PTU treatment was estimated chemically by measurement of plasma TSH and thyroxine (T_4) levels in the pups. On the fourth postnatal day the mean plasma TSH level in the experimental group was 31 mU per 100 ml compared to 4.7 mU per 100 ml in the control group. This indicated that a deficiency of peripheral thyroid hormone was causing a positive feedback at the hypophyseal level. The mild nature of the hypothyroidism was evidenced by the fact that on the twelfth postnatal day the mean plasma T_4 level of PTU treated pups was 3.6 μ g per 100 ml which was 70 percent of that of the controls (5.1 μ g per 100 ml). The plasma T_4 level of the undernourished animals was very similar to that of the hypothyroid group being 3.4 μ g per 100 ml on day 12 but interestingly their plasma TSH level

was also reduced being 4.9 mU per 100 ml compared with 10.1 mU per 100 ml in the control group. The plasma TSH levels of the hypothyroid animals on day 12 are not reported, but presumably they remained higher than those of the controls. The possibility that reduction in T_4 levels might result from lowered plasma concentrations of thyroid hormone binding capacity was considered and excluded by the demonstration that T_3 resin tests gave a lower result using pooled plasma from the underfed animals than from controls.

The low plasma TSH levels of underfed pups was investigated by incubating the pituitaries of underfed and control pups in vitro under control conditions and in the presence of 25 or 50 pg TRH per milliliter. TSH in the incubation medium was measured subsequently and TSH secretion was expressed as the difference between the release during a one hour incubation in the presence of TRH and that in the immediately preceding control hour. The weight of the pituitary from the underfed animals was approximately half that of the fed controls, being 0.37 ± 0.04 mg compared with 0.68 ± 0.08 mg. For this reason the in vitro incubations contained two experimental or one control gland.

TRH at 25 pg per milliliter concentration caused a modest stimulation of TSH release but at 50 pg per milliliter the effect was much greater. The TSH response to 50 pg TRH per milliliter was 2.8 U per milligram wet weight per hour from the control animals which was similar to that from the underfed, 2.6. It is impossible to calculate what increment above resting release rate this represented because of the method of presentation of results. The authors deduced from this finding that the pituitaries of the underfed pups had the same capacity to release TSH as those from the control group. This must be viewed with reserve, however, until the nature of the pituitary weight reduction of the experimental group is more clearly defined.

The finding of a normal capacity for TSH secretion in vitro led the authors to study next the hypothalamic TRH content.

This was done by dissecting blocks of the brain which were bounded anteriorly by the optic chiasma, laterally by the hypothalamic sulci, posteriorly by the mamillary bodies, and which extended 4 mm dorsally from the ventral surface of the median eminence. TRH was extracted from the block with methanol and the hypothalamic protein content was also measured. The hypothalamic TRH content of the control pups increased five-fold by the sixteenth day of postnatal life when the mean value was 2.68 ng. In the underfed pups the level was significantly less from day eight onwards whether expressed per hypothalamus or per milligram of hypothalamic protein. This last observation demonstrated that the TRH concentration was not reduced because of less total hypothalamic mass or a nonspecific reduction of protein synthesis.

The findings of Shambaugh and Wilber are noteworthy because they provide the first direct evidence for a cerebral origin of the hypothyroidism that accompanies undernutrition. The mechanism by which reduced TRH formation and presumably secretion occurs awaits further study. It is interesting, however, to observe that the modest hypothyroidism of the PTU treated pups which produced similar T_4 levels to that of the underfed animals resulted in a much smaller deficit in weight gain. Further work should also make it possible to state with confidence if this weight deficit is directly due to low circulating levels of T_4 or other experimental variables.

A recent detailed clinical study⁴ of thyroidal function in malnourished infants covers some of the same ground as the rat work and it is worthwhile to consider the two studies together. The children were of Mestizo origin from Peru. Six males with marasmus were studied within 24 hours of hospital admission at the age of five to ten months; five were studied again following partial rehabilitation of 24 to 76 days with a balanced diet of 125 to 175 kcal per kilogram per day; the sixth dying from septicemia. All were examples of severe marasmus with normal serum protein

levels. Blood was collected after an overnight fast the day after admission and just prior to discharge. The serum concentrations of T_4 , free T_4 (FT_4), thyroxine binding globulin (TBG), thyroxine binding pre-albumin (TBPA), and TSH concentration were measured on stored frozen samples. Control values were obtained from a group of 13 male siblings who were admitted to the hospital in good health before the age of three months and stayed up to two years, growing normally. Twenty-seven blood samples taken from these children at the ages of three to 40 months were used to construct curves of normal serum thyroid hormone ranges. This was important as normal thyroid hormone concentrations change markedly as a function of age in the early months of life,⁵ T_4 in particular falling with increasing age. On admission the marasmic children had a mean serum T_4 level of 9.5 μg per 100 ml which was significantly less than that of controls of comparable age of height (12.3 μg per 100 ml). Serum TBG, TBPA, and FT_4 levels were not significantly different from those of controls and all but one of the TSH values were below the sensitivity of the assay. No remarkable differences were noted following recovery.

Seven children with marasmic kwashiorkor were studied similarly. They were stunted and had the classical features of edema, hepatomegaly, skin and hair changes. On admission they were hypoproteinemic and anemic. Treatment included blood transfusion as well as nutritional rehabilitation. At the time of initial study they had low T_4 and TBG levels and normal or high TBPA and FT_4 levels. In four, the FT_4 level was above the normal range. TSH levels were normal, being measurable in six. After 22 to 41 days treatment the TBG levels had returned to normal and T_4 levels had risen but were still low. The TBPA values remained raised and the FT_4 fell to a significantly low mean. In five of the six in whom TSH had been detectable on admission there was a fall on recovery.

This study has confirmed the earlier report of F. Varga and B. Mess⁶ who, using a bioassay, had found low plasma and pituitary TSH concentrations in marasmus and kwashiorkor. These findings fit well with those from the rat experiments especially when it is recollected that marasmic infants have a normal thyroid hormone response to an injection of exogenous TSH.⁷ It seems reasonable to propose that the malnourished infant, with marasmus or kwashiorkor, like the underfed rat, has depression of hypothalamo-hypophyseal-thyroid axis of central origin. Differences between marasmus and marasmic-kwashiorkor seem to be largely explicable by changes in plasma thyroid-binding protein concentration in the latter condition. In neither study was plasma triiodothyronine concentration (T_3) measured; it would, however, be surprising if such measurements were discordant with a series of animal and human studies which are noteworthy for their consistency.

Finally there remains the question of what effect early malnutrition may have on thyroid function in later life following recovery. D. Blackmore⁸ studied this with a rat model. Male pups were overfed or underfed from the second to eighth day of life by adjustment of litter size and restriction of access by the underfed pups to the mother to seven hours each day. From the ninth day to the time of weaning at 21 days both groups were allowed unlimited access to suckle. Between 30 to 35 days and 50 to 55 days of age some of the animals were injected with ^{131}I and sacrificed 24 hours later.

The underfed animals still had a weight deficit at 30 to 35 days and at 50 to 55 days which was paralleled by that of the thyroid gland. When thyroid weight was expressed as a fraction of body weight (milligram per 100 g), the fraction was significantly lower in the overfed animals at both ages. It must be remembered, however, that the overfed animals might have been fatter at the time of sacrificing so that body weight was not as appropriate a

denominator of thyroidal weight as lean body mass. The fraction of the injected radioactive iodide remaining in the thyroid after 24 hours was similar in the over- and underfed animals but slightly and significantly more in those overfed. Also, the serum protein iodine at 50 to 55 days was slightly but significantly greater in the overfed animals. The author concluded that a short period of undernutrition in the neonatal period can have prolonged effects on thyroidal function in the rat, although, as he points out, the biological as opposed to the statistical significance of these findings remains open to speculation. Even more conjectural is the relevance of these findings to human infantile malnutrition.

Several questions raised by this research are of great interest. How does the brain perceive the undernourished state so that thyroidal function is dampened, and is there a teleological interpretation to the depression of thyroidal activity in order to conserve fuel in short supply? □

1. R. D. G. Milner: Endocrine Adaptation to Malnutrition. *Nutrition Reviews* 30: 103-106, 1972

2. R. M. Bassiri and R. D. Utiger: The Preparation and Specificity of Antibody to Thyrotropin Releasing Hormone. *Endocrinology* 90: 722-727, 1972
3. G. E. Shambaugh III and J. F. Wilber: The Effect of Caloric Deprivation upon Thyroid Function in the Neonatal Rat. *Endocrinology* 94: 1145-1149, 1974
4. G. G. Graham, J. M. Baertl, G. Claeysen, R. Suskind, A. H. Greenberg, R. G. Thompson, and R. M. Blizzard: Thyroid Hormonal Studies in Normal and Severely Malnourished Infants and Small Children. *J. Pediat.* 83: 321-331, 1973
5. M. T. O'Halloran and H. L. Webster: Thyroid Function Assays in Infants. *J. Pediat.* 81: 916-919, 1972
6. F. Varga and B. Mess: Serum Thyrotrophin in Semistarvation. *Acta Paediat. Acad. Sci. (Hung.)* 9: 197-203, 1968
7. F. Beas, F. Monckeberg and I. Horwitz: The Response of the Thyroid Gland to Thyroid Stimulating Hormone (TSH) in Infants with Malnutrition. *Pediatrics* 38: 1003-1008, 1966.
8. D. Blackmore: Effect of Early Postnatal Starvation on Subsequent Thyroid Function. *Biol. Neonat.* 23: 359-365, 1973

CELLULAR DEVELOPMENT AFTER FETAL THYROIDECTOMY

Thyroidectomy of the sheep fetus causes complex disturbances in the patterns of cellular development of various organs and particularly in the brain.

Key Words: fetal thyroidectomy, cellular growth, placental permeability, thyroxine, triiodothyronine

The growth of the ovine fetus after thyroidectomy in utero has been reviewed recently in this journal.¹ Thyroidectomy impairs fetal growth in the sheep as it does in man but the stunting is not severe and questions unresolved at the time of the last review related to the possible role of maternal thyroid hormones in fetal development and the nature of the growth deficit in cellular terms. Some of these points have since been clarified. A. Erenberg and his colleagues studied the

effects of fetal thyroidectomy on both materno-fetal thyroid interrelationships² and the cellular growth of the fetus.³

In the first report² the changes in fetal and maternal iodothyronine kinetics were studied after fetal thyroidectomy of seven lambs at 90 to 125 days gestation which is early in the third trimester. In four of the animals, indwelling catheters in the fetal carotid artery and maternal jugular vein were inserted at the time of operation. In the other three animals, the catheters were placed at a second operation three to four weeks later. After thyroidectomy the maternal plasma thyroxine (T₄) level re-

mained unchanged whereas the fetal plasma T_4 concentration fell exponentially from a mean level of 12.2 μg per 100 ml preoperatively to 1.7 μg per 100 ml three days later and became undetectable ($< 0.7 \mu\text{g}$ per 100 ml) by the fifth day. Kinetic studies involving injections of ^{125}I and ^{131}I iodothyronine hormones were performed four to 37 days post-thyroidectomy, by injecting T_4 or triiodothyronine (T_3) labeled with ^{131}I or ^{125}I into mother and fetus respectively at the same time. From these it was possible to calculate the mean $t_{1/2}$ for T_4 in mother and fetus to be 1.42 and 0.99 days and the T_4 fractional degradation rates to be 0.50 and 0.77 percent respectively. Placental transfer of T_4 occurred in both directions, was slight, but was greater from fetus to mother than vice versa.

Maternal T_3 levels were 94 ± 5.3 ng per 100 ml before operation and like T_4 did not change afterwards. Fetal T_3 was undetectable before and after operation. After the injection of radioactive T_3 the mean maternal $t_{1/2}$ and fetal $t_{1/2}$ were 6.7 and 10 hours and the fractional degradation rates, 2.52 and 2.67 percent respectively. Like T_4 a small transfer of T_3 occurred across the placenta in both directions but more from fetus to mother than from mother to fetus. Assuming that T_3 is four times as active biologically as T_4 the net equivalent turnover of T_4 was calculated as the turnover of $T_4 + 4T_3$. Using this expression the authors calculated that the equivalent turnover of T_4 in the thyroidectomized fetus was 7 percent of that occurring in euthyroid fetuses.⁴ Another consequence of thyroidectomy was the finding of greatly raised fetal thyrotropin levels in three animals. Overall the authors found that the athyroid fetus develops in the absence of significant quantities of thyroid hormones. Another possibility is that fetal tissues are much more sensitive to T_4 and that exposure to 7 percent of the normal levels still has an appreciable growth promoting effect.

The consequences of fetal thyroidectomy were considered by the same

group of workers in their other contribution.³ Five fetuses were thyroidectomized at 90 to 110 days gestation and were delivered 19 to 43 days later. The body and various organs were weighed, limb roentgenograms performed, and deoxyribonucleic acid (DNA), ribonucleic acid (RNA), and protein concentrations of selected organs were estimated. Control values for body and organ weight were obtained from 60 other fetuses on which surgical procedures had been performed with no intention of producing growth retardation. Normal values for the chemical estimates of growth were made from eight fetuses matched for sex and gestational age.

At the time of sacrificing the thyroidectomized fetuses, the plasma levels of T_4 and T_3 were immeasurable while TSH levels were very high, confirming that the animals were chemically hypothyroid. This was confirmed by careful inspection at necropsy. The fetuses tended to be small and light for their gestational age. The lung weights were significantly low whereas liver, kidney, heart, and brain weights were within normal limits.

The DNA/weight ratio was used as an index of cell concentration and the protein/DNA ratio as one of cell size. The small numbers of control and experimental observations may have hidden some differences which would have been significant had larger numbers been studied. No significant difference was noted in the concentration of RNA in any organ studied. In muscle, the source of which was not specified, there was a marked reduction in the cellular concentration but no change in cell size. The lungs of the thyroidectomized fetus were lighter because they contained a significantly lower protein/DNA ratio and an appreciable though not significant reduction in total DNA. They were thus low in both cell number and cell size. The protein/DNA ratio of the liver from the experimental animals was only 60 percent that of the control fetuses but the difference was not significant. As the fetal liver is composed of both hepatic and hemopoietic tissue it is possible that retardation of

growth of one of these could be masked by the normal development of the other. No difference in cell size or number was noted in the thymus, spleen, kidneys, or heart. In the brain different effects were observed in the cerebellum and cerebrum. The cerebellum of the thyroidectomized fetuses had a low protein/DNA ratio whereas that of the cerebrum was greater, though in neither case was the difference significant.

The radiographs showed fewer and smaller epiphyseal centers and a sclerotic appearance of the long bones. The authors also observed that there appeared to be retardation of wool follicle development, a lack of subcutaneous fat, and friability of striped muscle, confirming the earlier observations of P. S. Hopkins and G. D. Thorburn.⁵

Lipid analyses of the brain showed that the total cerebral lipid expressed as a fraction of the wet weight was significantly less in the thyroidectomized animals but when the different subfractions, namely phospholipids, cholesterol, cerebrosides, and sulfatides were examined individually, the levels were very similar. Similar findings came from analyses of the cerebellum with the exception that the difference in total lipid concentration failed to achieve significance.

The brain changes in the thyroidectomized fetal lamb may be compared with those in similar experiments using macaque monkeys (*Macaca mulatta*).⁶ Six fetuses were made cretinous by injecting the mothers with ¹³¹I at 71 to 88 days gestation which is approximately mid-pregnancy. The experimental animals and an equal number of controls were delivered by Cesarean section at 150 days. The injection made the mothers mildly hypothyroid as judged by measurement of serum T₄ levels but caused complete arrest of fetal thyroidal development since the thyroid could not be recognized at dissection.

Similar effects were found in the cerebrum and cerebellum of the experimental animals but were more marked in the cerebellum in which there was 10 percent

reduction in both weight and DNA content. The protein/DNA ratio was more reduced in the cerebrum than in the cerebellum. The reduction in total protein, nonprotein dry solid, and RNA was significant in both cerebrum and cerebellum. Two neuronal constituents measured, namely lipid N-acetylneuraminic acid (lipid NANA) and total sodium plus potassium ATPase activity, were each significantly reduced in cerebrum and cerebellum as were two markers of extraneuronal development, cholesterol and carbonic anhydrase. These prenatal changes in the monkey confirm findings made in the rat thyroidectomized at birth and are probably indicative of what is likely to happen in the human athyrotic fetus.

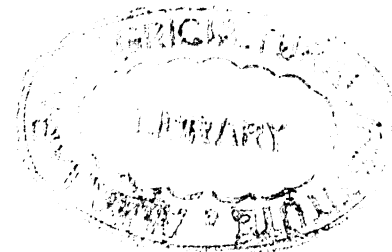
These studies complement the earlier reports of fetal thyroidectomy by illustrating the complexity of maternal and fetal thyroid hormone interrelationships and the diverse actions of fetal thyroid hormone lack on fetal development. It is clear that body weight or any other single anthropometric measurement is a very crude way of assessing fetal growth. An illustration of the difficulty of interpretation is given by the ovine fetal muscle. There was a similar protein/DNA concentration in the thyroidectomized and control fetuses but a significantly lower DNA concentration. What made up the balance of the weight—water, fat, or something else? Had a representative muscle, red or white, been removed carefully, weighed, and analyzed in more detail this fundamental point could have been resolved.

It is clear that much fetal development occurs independently of thyroid influence or that a little, about 7 percent, of the normal fetal thyroid hormone production goes a long way in its biological effect. The complete removal of thyroid hormones from the fetal circulation will be hard to achieve and this question may remain unanswered. Functionally, the most important is the effect on brain development. It is clear from studies on the rat, sheep, and monkey that brain growth in terms of both neuronal and glial development is

importantly influenced by fetal thyroid hormone lack at the time of the brain growth spurt.⁷ For species, including man, in which this takes place prenatally, the effect on the fetus of ingesting goitrogens or antithyroid drugs may be permanent even though apparent recovery appears to take place. □

-
1. Thyroid Hormones and Fetal Growth. *Nutrition Reviews* 31: 67-70, 1973
 2. A. Erenberg, K. Omori, W. Oh, and D. A. Fisher: The Effect of Fetal Thyroidectomy on Thyroid Hormone Metabolism in Maternal and Fetal Sheep. *Pediat. Res.* 7: 870-877, 1973
 3. A. Erenberg, K. Omori, J. H. Menkes, W. Oh, and D. A. Fisher: Growth and Development of the Thyroidectomized Ovine Fetus. *Pediat. Res.* 8: 783-789, 1974
 4. D. A. Fisher, J. H. Dussault, A. Erenberg and R. W. Lam: Thyroxine and Triiodothyronine Metabolism in Maternal and Fetal Sheep. *Pediat. Res.* 6: 894-899, 1972
 5. P. S. Hopkins and G. D. Thorburn: The Effects of Fetal Thyroidectomy on the Development of the Ovine Fetus. *J. Endocrinol.* 54: 55-66, 1972
 6. A. B. Holt, D. B. Cheek and G. R. Kerr: Prenatal Hypothyroidism and Brain Composition in a Primate. *Nature* 243: 413-415, 1973
 7. J. Dobbing: Undernutrition and the Developing Brain: The Relevance of Animal Models to the Human Problem. *Am. J. Dis. Child.* 120: 411-415, 1970
-

NUTRITION NOTES



Recent Books

Essentials of Food and Nutrition by M. Swaminathan. Vol. I—Fundamental Aspects; Vol. II—Applied Aspects. Copies available in the United States from M. S. Venkatesan, Ph.D, P.E., P. O. Box 3905, Fullerton, California, 92631. Vol. I, pp. 576, price \$10.00; Vol. II, pp. 515, price \$8.00.

Toxicological Evaluation of Certain Food Additives with a Review of General Principles and of Specifications, Seventeenth Report of the Joint FAO/WHO Expert Committee on Food Additives. WHO Technical Report Series, 1974, No. 539. Published by FAO and WHO. Available from World Health Organization, Distri-

bution and Sales Service, 1211 Geneva 27, Switzerland. Pp. 40. Price Sw. fr. 5.

Skeletal Maturity of Children 6-11 Years, United States, Vital and Health Statistics, Series 11, No. 140, DHEW Publication No. (HRA) 75-1622. Available from the Superintendent of Documents, U. S. Government Printing Office, Washington, D. C. Pp. 62. Price \$1.25.

Morinda: An Economic Analysis of Malnutrition Among Young Children in Rural India, by F. J. Levinson. Published by Cornell/MIT International Nutrition Policy Series, E53-471, 30 Wadsworth Street, Cambridge, Massachusetts 02139. Pp. 97. Price \$2.75 (prepaid).

Meeting Announcements

Symposium on Specifications for Food Chemicals

A symposium on Specifications for Food Chemicals will be held in the auditorium of the National Academy of Sciences in Washington, D. C. on March 13. The program is free and open to the public. During the day-long program, national and international activities and problems in the development and application of food chemical specifications will be discussed.

Opening remarks by Dr. William J. Darby, president of the Nutrition Foundation, will be followed by a critique of the current Food Chemicals Codex specifications and a discussion of procedures for updating standards. The remainder of the morning session will be devoted to four reports by FDA spokesmen and others

dealing with regulatory trends and manufacturing processes affecting food chemicals. A question and answer session will follow each report.

Keynote speaker for the afternoon session will be G. F. Wilmink, cabinet adviser, Netherlands Ministry of Food and Agriculture. Dr. Wilmink will discuss the activities of the Joint FAO/WHO Codex Committee on Food Additives. Dr. George W. Irving, Jr., of the Select Committee on GRAS Substances of the Federation of American Societies for Experimental Biology will discuss the relationship of specifications to safety evaluations of GRAS substances.

The Academy's Subcommittee for the

Food Chemicals Codex, the organizing body for the symposium, will hold an open meeting the day following the symposium, March 14. Final arrangements for this meeting will be announced during the symposium.

Proceedings of the symposium will be published later this year.

For further information, contact Durward F. Dodgen, Foods Chemicals Codex, National Academy of Sciences, 2101 Constitution Avenue, N. W., Washington, D. C. 20418. □

International Plenary Session of FOOD-MAN-SOCIETY

The third meeting of the International Organization for the Study of Human Development will be held in Madrid, Spain, September 22-24, 1975. Non-members are invited. For further information contact:

Executive Secretary
D. N. Walcher, M.D.
Human Development Building
University Park, Pennsylvania 16802

New

Unique

INDEX OF NUTRITION EDUCATION MATERIALS

The Index of Nutrition Education Materials has been prepared by the Office of Education of the Nutrition Foundation to assist teachers and the general public in acquiring useful teaching aids and educational materials.

The Index lists more than 1,000 booklets, pamphlets, audio-visual aids and lists of recommended books available from federal agencies, professional societies, and educational organizations or foundations. Other sources for education material are included.

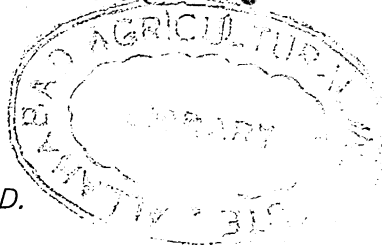
Gives addresses of important local contacts and sources in each state.

Source, subject matter, readership level and description are given for each publication in color-coded sections for easy reference. \$5.00 postpaid

Order from:

OFFICE OF EDUCATION
THE NUTRITION FOUNDATION
888 Seventeenth Street, N.W.
Washington, D. C. 20006

23 JUL 1975



Biotin

by Donald B. McCormick, Ph.D.

In the years following the recognition of biotin as a vitamin for mammals and many simpler organisms, evidence accumulated for its participation in carboxylation reactions. The importance of biotin in fatty acid biosynthesis is underscored by its occurrence as a functional component in acetyl-CoA carboxylase, the enzyme first identified as containing the vitamin as a prosthetic group.¹ Through the work of F. Lynen,² J. Knappe,³ and their colleagues during the 1960's, the involvement of biotin, covalently attached as an amide through the ϵ -amino lysyl residues of carboxylating enzymes, was largely elucidated. It has become clear that all biotin-dependent carboxylases, including a transcarboxylase, use the biotinyl moiety as a mobile carboxyl carrier to vector this group to specific acyl-CoA intermediates. In an interesting variation of this theme, a biotin-containing ATP:urea amidolyase effects the cleavage of urea in certain microorganisms by first catalyzing N-carboxylation of the substrate.⁴

The way in which the biotin becomes covalently appended to an enzyme has been elucidated for several systems.⁵ In the first step, biotinyl adenylate is formed

from biotin and ATP as the Mg^{2+} chelate. In the second step, the biotinyl moiety is transferred to the apocarboxylase subunit in a synthetase-catalyzed reaction to form the holocarboxylase.

Lynen and his co-workers isolated the 1'-N-carbomethoxy derivative of biotin methyl ester, identical to that obtained by chemical synthesis, from beta-methylcrotonyl-CoA carboxylase in which a second molecule of noncovalent biotin can be carboxylated by the ATP-dependent reaction with bicarbonate.^{6,7} Further evidence that the ureido 1'-nitrogen of biotin, which is opposite the side chain, is the site of carboxylation was supplied by isolating the same dimethyl ester of carboxybiotin after diazomethane treatment and proteolysis of enzymes that contained the covalently attached carboxylated vitamin.⁸ The possibility had been raised from studies with models that if carboxylation were to occur at the ureido oxygen, methylation would facilitate O to N migration of the methoxycarbonyl group and lead to the same derivative, viz. 1'-N-carbomethoxy-D-biotin methyl ester.⁹ However, M. D. Lane and his co-workers¹⁰ provided unequivocal evidence that it is only the 1'-N-carboxybiotinyl moiety that functions in the acetyl-CoA carboxylase system from *Escherichia coli*.

The means by which CO_2 (as HCO_3^-) and biotin (as biotinyl enzyme) react to form the 1'-N-carboxybiotinyl compound is less well established. Suggestive evidence has been obtained that this ATP-dependent process results in the intermediate formation of ADP and carbonyl phosphate.¹¹

Dr. McCormick is Professor of Biochemistry and Nutrition at Cornell University, Division of Nutritional Sciences, Savage Hall, Ithaca, New York 14850.

A limited number of reprints of this article may be obtained from the author. THERE ARE NO REPRINTS OF UNSIGNED REVIEWS.

Carboxylase systems have been shown typically to consist of subunits involved with the formation and transference of the carboxyl function from the biotin-containing subunit. The three protein components, biotin carboxylase (BC), carboxyl-transferase (CT), and biotin-containing carboxyl carrier protein (CCP), of the acetyl-CoA carboxylase system have been resolved and purified from cell-free extracts of *E. coli* B.¹⁵ The overall operation of such a typical carboxylase can be visualized as shown in Figure 1.

The regulatory aspects of carboxylases are the important with respect to the nutrition of the organism. Pyruvate carboxylase and acetyl-CoA carboxylase, both essential to the metabolism of man and numerous other organisms, are subject to allosteric regulation. The mitochondrial pyruvate carboxylase is activated by catalytic amounts of acetyl-CoA,^{8,16,17} so that as more carbohydrate is glycolyzed to pyruvate and enters the mitochondria where some of it is decarboxylated to form acetyl-CoA, more of the remaining pyruvate is then carboxylated to form oxaloacetate. The increasing concentrations of acetyl-CoA and oxaloacetate, both derived from pyruvate, lead to greater production of citrate. Some of the mitochondrial citrate, that fraction over what can be catabolized in the tricarboxylic acid cycle, can diffuse back into the cytoplasm and activate acetyl-CoA carboxylase^{8,18} to produce malonyl-CoA and, eventually, more fatty acids and fat. These important relationships can be summarized as shown in Figure 2.

Figure 1

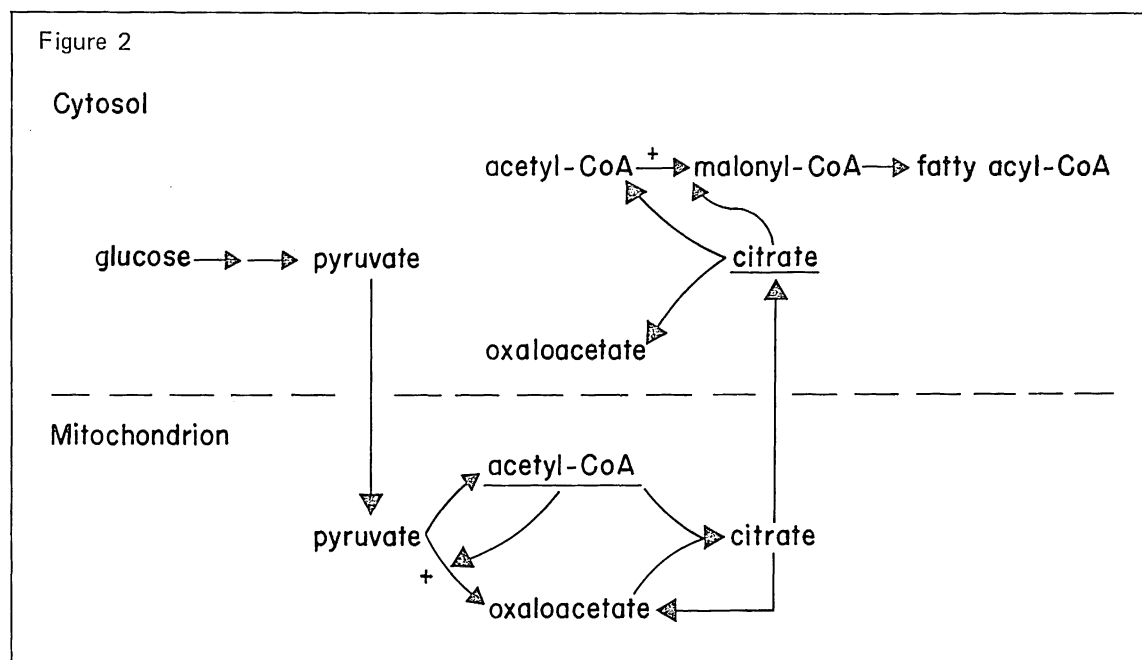
The diagram illustrates the formation of a cyclic intermediate from HCO_3^- and ATP, catalyzed by BC-Catalyzed and CT-Catalyzed pathways. The reaction involves the formation of a cyclic intermediate, which is then converted to a cyclic intermediate, and finally to a cyclic intermediate.

The reaction scheme shows the following steps:

- $\text{HCO}_3^- + \text{ATP} + (\text{CH}_2)_4$ reacts to form a cyclic intermediate (labeled BC-Catalyzed).
- The cyclic intermediate is converted to a cyclic intermediate (labeled CT-Catalyzed).
- The final product is a cyclic intermediate, which is then converted to a cyclic intermediate.

The diagram includes chemical structures for the reactants, intermediates, and products, as well as the catalytic pathways (BC-Catalyzed and CT-Catalyzed).

Figure 2



A further control on this process is that malonyl-CoA and fatty acyl-CoA derivatives are feedback inhibitors by virtue of their action as negative effectors of the acetyl-CoA carboxylase.¹⁹

Another carboxylase that deserves special mention is the enzyme that catalyzes carboxylation of propionyl-CoA to methylmalonyl-CoA.²⁰ Propionate utilization in animals, and especially in certain rumen microflora, is an important part of their metabolism.

Biosynthesis

The biosynthesis of biotin is now understood in considerable detail. As this subject has been reviewed recently,²¹ only the outlines of present knowledge will be given here. Most microorganisms capable of biosynthesizing the vitamin appear to utilize a pathway from pimelic acid through 7,8-substituted pelargonic acids and dethiobiotin prior to incorporation of sulfur to form biotin. However, one strain of *Achromobacter* was reported to incorporate the elements of cysteine, together with pimelyl-CoA, to form the vitamin from a sulfhydryl precursor.²² The intermediates and enzymes responsible for catalyzing the steps to dethiobiotin have been

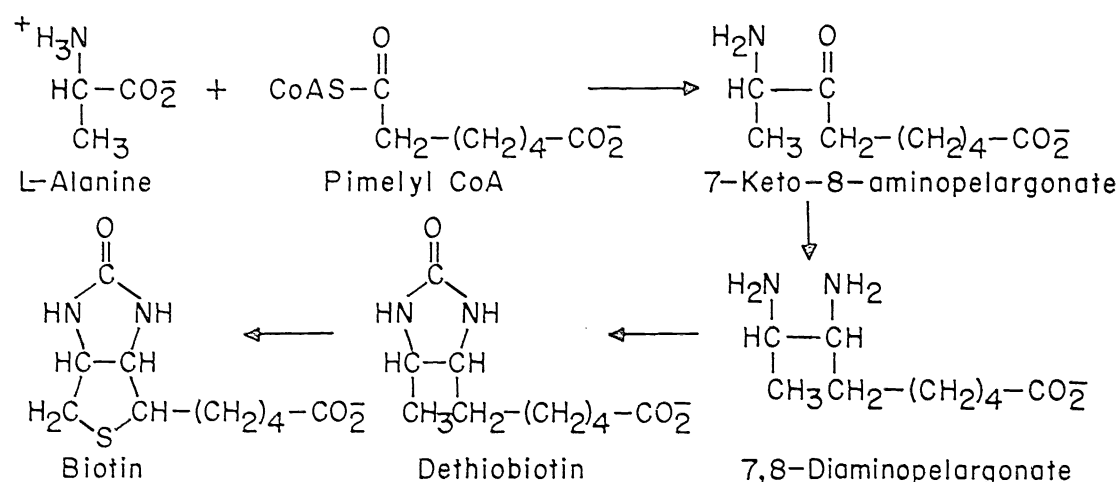
fairly well characterized and controls that operate noted. This pathway is shown in Figure 3.

The means by which dethiobiotin is converted to biotin has not been established, but it undoubtedly involves more than one step. By the use of radioactive dethiobiotin labeled with ¹⁴C or ³H in different positions, it has been proved that the skeleton of this precursor is incorporated intact into biotin formed in *Aspergillus niger*.²³ It would also seem that four or more hydrogens must be abstracted from dethiobiotin in this conversion.

Transport and Metabolism

The ways in which biotin, the sulfoxides, sulfone, and dethio derivative are transported and metabolized has been investigated by K. Ogata and by D. B. McCormick, L. D. Wright, and their co-workers.²⁴⁻³⁶ The uptake^{29,30} and metabolism²⁵⁻³² of biotin and its analogs by a pseudomonad, which can use the vitamin as a sole source of C, N, S, and energy, have been investigated with radioactive compounds and most of the primary metabolites isolated and chemically characterized. The primary events and their general

Figure 3

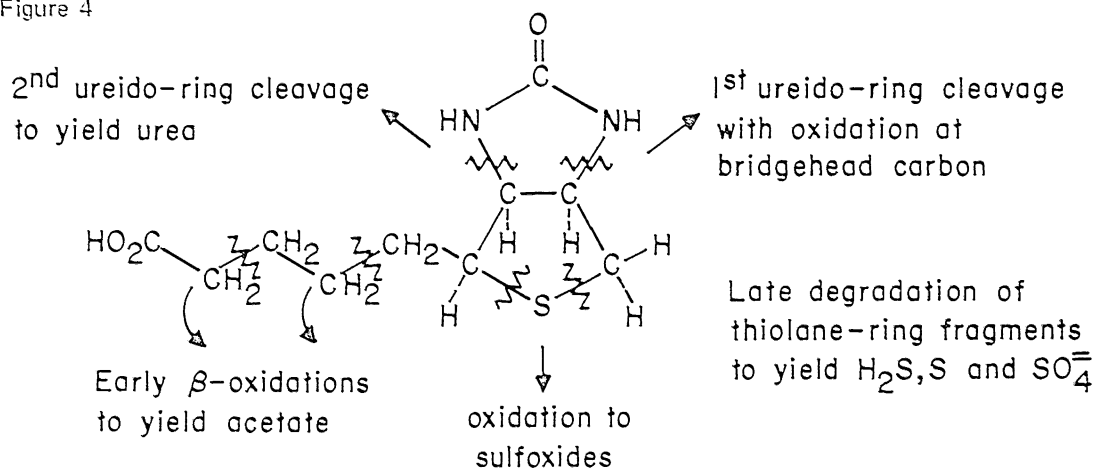


sequence in the total degradation of biotin are indicated in Figure 4.

Much of the carbon needed for growth of the microorganism is derived from metabolizable acetate by successive beta-oxidative cleavage of the valeric acid side chain to form the two-carbon shorter bisnorbiotin, and then the four-carbon shorter tetranorbiotin.²⁸ The acid side chains of dethiobiotin^{27,33} and the unnatural homobiotin²⁷ are similarly beta-oxidized in this microorganism, as well as others. The intermediates expected at each stage of this process, viz. the alpha, beta-unsaturated, beta-keto, and beta-hydroxy acids, have

been identified.²⁸⁻³¹ The ureido ring can be cleaved between the N and bridgehead C opposite the side chain of the bisnor catabolite.³² To some extent, hydrolysis of the carbamyl portion can occur, and following this, a fraction is recarbamylated and the ring reformed, both to regenerate the all *cis*-bisnorbiotin, as well as the *trans* counterpart. However, most of the nitrogen is released by a second cleavage of the ureido compound to form urea, which is then hydrolyzed to NH_4^+ and CO_2 .²⁸ Degradation of the remaining thiolane-ring fragments, not as yet isolated, produce inorganic sulfur at different oxidation-

Figure 4



reduction levels. Even earlier oxidation of the thioether sulfur of biotin leads to some formation of both the D- and L-sulfoxide.^{26,28} The D-isomer can be partially degraded but is largely catabolized after reductive reversion to biotin.³⁰ The L-isomer²⁹ and sulfone³¹ are more inert and are mainly converted to the beta-hydroxy side-chain metabolites, which accumulate in the culture medium.

The metabolism of biotin and excretion of the vitamin and catabolites from the rat have also been determined.^{34,35} In general, the mammal cannot significantly degrade the ring system of the vitamin but converts a small portion to the sulfoxides, which arise through microsomal mixed-function oxidase activity in liver. A larger fraction is degraded to bisnorbiotin, which results from beta-oxidation of the side chain effected in mitochondria. These compounds and traces of the methyl ketones, resulting from decarboxylation of the intermediate beta-keto acids, are excreted with unaltered free vitamin in the urine.

Deficiency

In spite of some catabolic loss of biotin in the body tissues, deficiencies do not occur frequently in the human and most animal species. The small amounts required are usually readily supplied by the diet and are further augmented in most species by microfloral synthesis. For these reasons, it is only after the feeding of raw egg white as a source of avidin to complex the biotin that deficiency symptoms appear. Even though the ingested complex is relatively stable and prevents biotin from being absorbed in its passage through the alimentary tract, the injected complex is slowly degraded and the vitamin released to be utilized and metabolized.³⁶ □

1. S. Wakil and D. M. Gibson, *Biochim. Biophys. Acta* 41: 122-129, 1960
2. F. Lynen, *Biochem. J.* 102: 381-400, 1967
3. J. Knappe, *Ann. Rev. Biochem.* 39: 757-776, 1970
4. R. J. Roon and B. Levenberg, *J. Biol. Chem.* 245: 4593-4595, 1970
5. P. N. Achuta Murthy and S. P. Mistry, *Biochem. Rev.* XLIII: 1-10, 1972
6. F. Lynen, J. Knappe, E. Lorch, G. Jütting, and E. Ringelmann, *Angew. Chem.* 71: 481-486, 1959
7. J. Knappe, H.-G. Schlegel, and F. Lynen, *Biochem. Z.* 335: 101-122, 1961
8. J. Moss and M. D. Lane, *Advances Enzym.* 35: 321-442, 1971
9. T. C. Bruice and A. F. Hegarty, *Proc. Nat. Acad. Sci. USA* 65: 805-809, 1970
10. R. B. Guchhait, S. E. Polakis, D. Hollis, C. Fenselau, and M. D. Lane, *J. Biol. Chem.* 249: 6646-6656, 1974
11. S. E. Polakis, R. B. Guchhait, E. E. Zwergel, M. D. Lane, and T. G. Cooper, *J. Biol. Chem.* 249: 6657-6667, 1974
12. A. F. Hegarty, T. C. Bruice, and S. J. Benkovic, *Chem. Commun.* 20: 1173-1174, 1969
13. D. B. McCormick, *J. Heterocyclic Chem.* 10: 235-237, 1973
14. R. Griesser, H. Sigel, L. D. Wright, and D. B. McCormick, *Biochemistry* 12: 1917-1922, 1973
15. R. B. Guchhait, S. E. Polakis, P. Dimroth, E. Stoll, J. Moss, and M. D. Lane, *J. Biol. Chem.* 249: 6633-6645, 1974
16. *Metabolic Regulation and Enzyme Action*. A. Sols and S. Grisolia, Editors, vol. 19, p. 53. Academic Press, New York, 1970
17. M. C. Scrutton, *J. Biol. Chem.* 249: 7057-7067, 1974
18. *Lipid Metabolism*. S. J. Wakil, Editor, p. 9. Academic Press, New York, 1970
19. S. Numa, W. M. Bortz, and F. Lynen, *Advances Enzym. Reg.* 3: 407-423, 1965
20. Y. Kaziro and S. Ochoa, *Advances Enzym.* 26: 283-378, 1964
21. M. A. Eisenberg, *Advances Enzym.* 38: 317-372, 1973
22. A. Lezius, E. Ringelmann, and F. Lynen, *Biochem. Z.* 336: 510-525, 1963
23. H. C. Li, D. B. McCormick, and L. D. Wright, *J. Biol. Chem.* 243: 6442-6445, 1968
24. D. B. McCormick and L. D. Wright in *Comprehensive Biochemistry*. M. Florkin and E. H. Stotz, Editors, vol. 21, pp. 81-110. Elsevier Publishing Co., Amsterdam, 1971
25. R. N. Brady, L. F. Li, D. B. McCormick, and L. D. Wright, *Biochem. Biophys. Res. Commun.* 19: 777-782, 1965
26. R. N. Brady, H. Ruis, D. B. McCormick, and L. D. Wright, *J. Biol. Chem.* 241: 4717-4721, 1966

27. H. Ruis, R. N. Brady, D. B. McCormick, and L. D. Wright, *J. Biol. Chem.* 243: 547-551, 1968
28. S. Iwahara, D. B. McCormick, L. D. Wright, and H. C. Li, *J. Biol. Chem.* 244: 1393-1398, 1969
29. J. A. Roth, D. B. McCormick, and L. D. Wright, *J. Biol. Chem.* 245: 6264-6268, 1970
30. W. B. Im, J. A. Roth, D. B. McCormick, and L. D. Wright, *J. Biol. Chem.* 245: 6269-6273, 1970
31. M. N. Kazarinoff, W. B. Im, J. A. Roth, D. B. McCormick, and L. D. Wright, *J. Biol. Chem.* 247: 75-83, 1972
32. W. B. Im, D. B. McCormick, and L. D. Wright, *J. Biol. Chem.* 248: 7798-7805, 1973
33. H. C. Li, D. B. McCormick, and L. D. Wright, *J. Biol. Chem.* 243: 4391-4395, 1968
34. H. M. Lee, L. D. Wright, and D. B. McCormick, *J. Nutrition* 102: 1453-1464, 1972
35. H. M. Lee, N. E. McCall, L. D. Wright, and D. B. McCormick, *Proc. Soc. Exp. Biol. Med.* 142: 642-644, 1973
36. H. M. Lee, L. D. Wright, and D. B. McCormick, *Proc. Soc. Exp. Biol. Med.* 142: 439-442, 1973

Nutrition Reviews

authoritative articles by distinguished international scientists • competent, critical, and knowledgeable reviews • interpretative summaries of important original clinical and experimental research • selected excerpts from classic publications marking milestones in nutrition research • informative special reports on government and organization actions • contributing editors review journals of widespread interest covering biochemistry, food science, public health, toxicology, dentistry, pediatrics, internal medicine, gastroenterology and other clinical specialties •

Send subscription and renewal requests to:

THE NUTRITION FOUNDATION
489 Fifth Avenue
New York, New York 10017

\$12.00 per year

\$6.00 for students

THE RELATIONSHIP BETWEEN INFECTION AND THE IRON STATUS OF AN INDIVIDUAL

Recent work on the incidence of infection and the defense mechanisms against infection in iron deficiency is discussed.

Key Words: iron deficiency, infection, immune mechanisms

There is a body of experimental and clinical evidence suggesting that the iron status of an individual may affect his resistance to infection. Thus iron-binding proteins such as transferrin and the conalbumin of egg-white, perhaps by reducing the availability of iron, may inhibit bacterial growth *in vitro*.^{1,2} Increased iron supply to tissues may predispose to infection, e.g. parenteral iron may exacerbate chronic pyelonephritis,³ and free blood in body fluids may enhance bacterial growth.⁴

It could therefore be postulated that states of increased iron availability in the blood might predispose to infection. Conversely, iron deficiency might protect the individual against infection. The situation is not yet clear. Patients with an iron overload due to hemochromatosis or multiple blood transfusions are not notably prone to infection. Many types of hemolytic anemia are also not thought to be predisposing. Individuals with sickle cell anemia are undoubtedly liable to *Salmonella* osteomyelitis but this may be at least partly due to the bone infarction which readily occurs in this disease.

The mechanisms by which a lack of available iron might influence conditions for bacterial growth are several. A direct lack of availability of iron in the blood or in the body fluids could inhibit proliferation of microorganisms. This situation is found in iron-lack anemia but a low serum

iron is also found in the presence of inflammation or infection *per se*. This could perhaps represent part of the body's defense mechanism against infection.

Other major factors in resistance to infection are, however, the reticulo-endothelial system including the polymorphonuclear leucocytes of the peripheral blood and cell-mediated immunity which depends on normal lymphocyte function. Previous work suggested that in iron deficiency, although leucocyte phagocytic capacity may be normal, killing capacity is reduced.⁵ The underlying mechanism may be impaired synthesis of iron-containing enzymes.⁶ D. H. M. Joynson and co-workers⁷ assessed peripheral blood lymphocyte function in iron deficiency. They found impairment of lymphocyte transformation with purified protein derivative and *Candida* antigen as the stimulating factors. The intradermal injection of these antigens also gave fewer positive delayed-hypersensitivity skin reactions than in an iron replete control group. The production of a macrophage inhibition factor was reduced when *Candida* antigen was the stimulus, but not when purified protein derivative was used.

A possible criticism of this work is that previous exposure to an antigen *in vivo* is necessary for lymphocytes to respond by transformation to the antigen *in vitro*. The usual measurement of previous exposure, delayed hypersensitivity reaction on skin testing, was depressed. This could imply impairment of this parameter with iron de-

iciency, but also non-exposure to the antigen. Restoration to normality of the in vitro tests after correction of the iron deficiency, would have been an important positive control.

More recent work by P. Kulapongs and his co-workers⁸ suggested, however, that there is no major defect in the function of lymphocytes or polymorphonuclear leucocytes in uncomplicated iron deficiency.

Studies were carried out in eight children with severe iron-deficiency anemia. In seven of these, hookworm infestation was a probable major etiological factor. Vitamin deficiencies were excluded by assay of serum vitamin B₁₂ and folate levels. Iron deficiency was documented by measurement of serum iron, iron-binding capacity levels, and assessment of bone marrow iron stores. Investigations were repeated after the restoration of normal hemoglobin levels consequent on the treatment with parenteral iron. Lymphocyte function was measured by assessing the response to stimulation with phytohemagglutinin (P.H.A.) of lymphocytes labeled with tritiated thymidine. The mean percent of lymphocyte transformation and proliferative index (incorporation of tritiated thymidine related to control unstimulated cultures) showed no significant difference. Similarly bactericidal activity of the polymorphonuclear leucocytes against *Escherichia coli* showed no significant difference in results when compared to those from the normal children. Only one individual in the test group had a subnormal leukocyte bactericidal activity when homologous serum was used in the test system, but normal values were obtained when autologous serum was used. This work, therefore, does not provide a pathogenetic basis for reports that iron-deficient individuals are more prone to infection.⁹

The obverse, that iron deficiency may protect against infection, is suggested by recent work in Dar-es-Salaam by A. E. J. Masawe and his colleagues.¹⁰ Successive anemic admissions to a hospital were categorized by full hematological work-up including the estimation of bone marrow iron

stores. One hundred and ten patients were classified as having one of the following varieties of anemia: iron-deficiency, megaloblastic, dimorphic (i.e. mixed iron deficiency and megaloblastic), hemolytic anemia, and refractory anemia due to chronic infection or inflammation.

Bacterial infections were found to be most common in hemolytic anemia (83 percent), refractory anemia (83 percent), and megaloblastic anemia (64 percent). The incidence in the 38 patients with pure iron deficiency was much lower and none of the 29 patients with dimorphic anemia had infection. Conversely 24 percent of the patients with iron deficiency, either alone or associated with megaloblastic anemia, had malaria but only 5 percent of the patients in the other three groups were so infected, the majority being in the dimorphic group. There was felt to be some relationship between iron therapy and the development of malaria. In spite of inadequate documentation in some cases, half the iron-deficient patients with malaria developed the infection shortly after iron therapy.

If this resistance of iron-deficient individuals to infection is confirmed by further larger studies, speculation as to the possible pathogenesis is of interest. There is evidence that the presence of iron aids bacterial growth¹¹ and the converse might be true. Alternatively transferrin itself, with the raised levels found in iron deficiency, might have a direct protective effect. Excess iron can abolish the bacteriostatic effect and antisera to certain microorganisms. It has also been suggested that unsaturated transferrin may be a necessary co-factor for such antisera.¹² Another possible pathogenetic mechanism is blockade and reduced phagocytic capacity of the reticulo-endothelial system in states of iron overload.¹³

There are obviously many factors to be considered and much experimental work remains to be done. The incidence of iron deficiency is so worldwide that its relationship to infection is of paramount importance. □

1. A. L. Schade and L. Caroline: Raw Hen Egg White and the Role of Iron in Growth Inhibition of *Shigella dysenteriae*, *Staphylococcus aureus*, *Escherichia coli* and *Saccharomyces cerevisiae*. *Science* 100: 14-15, 1944
2. A. L. Schade and L. Caroline: An Iron-Binding Component in Human Blood Plasma. *Science* 104: 340-341, 1946
3. J. Fletcher and E. Goldstein: The Effect of Parenteral Iron Preparations on Experimental Pyelonephritis. *Brit. J. Exp. Path.* 51: 280-285, 1970
4. G. H. Bornside, P. J. Bouis, Jr., and I. Cohn, Jr.: Hemoglobin and *Escherichia coli*, A Lethal Intraperitoneal Combination. *J. Bact.* 95: 1567-1571, 1968
5. R. K. Chandra: Reduced Bactericidal Capacity of Polymorphs in Iron Deficiency. *Arch. Dis. Child.* 48: 864-866, 1973
6. A. Jacobs: Tissue Changes in Iron Deficiency. *Brit. J. Haematol.* 16: 1-4, 1969
7. D. H. M. Joynson, D. M. Walker, A. Jacobs, and A. E. Dolby: Defects of Cell-Mediated Immunity in Patients with Iron Deficiency Anaemia. *Lancet* II: 1058-1059, 1972
8. P. Kulapongs, V. Vithayasai, R. Suskind, and R. E. Olson: Cell-Mediated Immunity and Phagocytosis and Killing Function in Children with Severe Iron-Deficiency Anaemia. *Lancet* II: 689-691, 1974
9. Nutritional Anaemias. World Health Organization Technical Report Series No. 405: 13-21, 1968
10. A. E. J. Masawe, J. M. Muindi, and G. B. R. Swai: Infections in Iron Deficiency and Other Types of Anaemia in the Tropics. *Lancet* II: 314-317, 1974
11. J. J. Bullen, A. B. Wilson, G. H. Cushnie, and H. J. Rogers: The Abolition of the Protective Effect of *Pasteurella septica* Antiserum by Iron Compounds. *Immunology* 14: 889-898, 1968
12. H. J. Rogers: Bacteriostatic Effects of Horse Sera and Serum Fractions on *Clostridium welchii* Type A, and the Abolition of Bacteriostasis by Iron Salts. *Immunology* 12: 285-301, 1967
13. E. R. Gabrieli and H. Holmgren: Studies in the Blockade of the Reticulo-Endothelial System. *Acta Path. Microbiol. Scandinav.* 31: 205-211, 1952

RENAL HANDLING OF SALT BY PRETERM INFANTS

Preterm infants respond to an oral sodium load with a greater natriuresis than term infants. The postnatal development of renal sodium handling by these infants does not differ from that which would have occurred in utero.

Key Words: preterm infants, renal function, sodium chloride, glomerular filtration rate

The development of milk formulas for newborn infants has a long history. Most of the components of milk have been studied intensively and modified in form and concentration considerably in various preparations offered to the public. Many of the alterations made have been done so on the empirical basis of "humanizing" cow's milk, the assumption being that human breast milk is the best formula for infant development and the closer a manufactured product can be made to resemble it, the better that formula will be. This approach is laudable but somewhat naive, especially

when the needs of preterm infants have to be considered. These babies have to be nourished with milk for up to 12 weeks before they would have first ingested the milk had they been born at term. In considering the needs of preterm infants attention must be given not only to the immaturity of certain digestive, absorptive, and excretory functions at the time of birth but also the effects of extrauterine life on the ontogeny of these functions. The effects of premature birth on the gastrointestinal tract have been reviewed here recently.¹ The purpose of this review is to draw attention to recent developments in the renal handling of sodium by preterm infants. This work is important because

changes have been made in the solute content of formulas recently on the basis of the inability of the neonatal kidney to handle sodium.

A. Aperia and her colleagues² studied the renal excretion of sodium in newborn infants of varying gestational ages during the first week of life or at intervals thereafter. The preterm infants were studied between the ages of two to three weeks or when they were equivalent to 40 weeks gestational age. All infants were fed breast milk or cow's milk formula to give 120 to 130 kcal per kilogram of body weight per 24 hours. The test consisted of infusing breast milk or formula diluted three-fold via a nasogastric tube. A volume corresponding to 2 percent of the body weight was given over 60 minutes followed by a quarter of this each succeeding 30 minutes. After 90 to 120 minutes fluid loading a salt load of 0.12 g of sodium chloride per kilogram of body weight was given as a 1 percent saline solution in the diluted formula. All urine voided over the next five hours was collected. Altogether, 49 such studies were performed in 44 infants of 29 to 37 weeks gestational age and normal birth weight. The infants were divided into three groups: 15 of 29 to 33 weeks (group I), 16 of 34 to 35 weeks (group II), and 13 of 36 to 37 weeks (group III). A fourth group of 20 term infants which had been studied in the same way and reported earlier³ was included for comparison. In 17 preterm infants and three term infants an estimate of the glomerular filtration rate (GFR) was made by injecting inulin intravenously in the latter part of the sodium load test and measuring inulin in capillary blood and urine thereafter.

All infants responded to the sodium load with an increase in urinary sodium excretion. In the term group the pattern of excretion was reproducible³ and similar to but less than that occurring in older children with an increase in the hourly sodium excretion in the second hour of study. This was followed by a high, steady rate of sodium loss up to the end of the fifth hour. In the preterm infants the response was

more variable, with fluctuation between periods being noted.

A comparison of the different gestational age groups during the first week of life showed that they all had similar hematocrits. Groups I and II had a similar and slightly lower mean GFR than groups III and IV. A more surprising finding was that the infants of 29 to 35 weeks gestational age had significantly higher mean hourly sodium excretion rates of 3 to 4 mEq per 1.73 m^2 per hour than did those of 36 to 40 weeks in whom the mean rate was 1 to 2 mEq per 1.73 m^2 per hour. Both preterm and term infants had similar urine diluting capacities as estimated by the relationship between the free water clearance and the sum of the sodium and free water clearances.

Increasing postnatal age was associated with a fall in hematocrit which was greatest in the most premature group. A slight fall in serum albumin was noted in groups I and II as they reached postnatal ages equivalent to 40 weeks gestational age. No statement about the significance of these changes is made and their interpretation is difficult because all the infants except three were studied only once, and the changes occurring as a function of postnatal age were made cross-sectionally on small groups. Serum sodium concentration remained constant in all groups at all ages studied. The glomerular filtration rate of the preterm groups increased with postnatal age. Their mean urinary sodium excretion rate, however, fell to levels characteristic of term infants in the first postnatal week.

The low GFR of the preterm infants shortly after birth is partly due to the histologic immaturity of glomerular development which is not complete until the thirty-sixth week⁴ and partly to the low hydrostatic pressure perfusing the kidneys.⁵ Their relatively good natriuretic response to a salt load is somewhat unexpected until it is appreciated that this is the consequence of another immaturity, in this case of tubular sodium reabsorption. Sodium loss is greater from the preterm newborn because inability to reabsorb

more than counterbalances the low delivery of salt to the renal tubule. Furthermore, urinary sodium excretion is enhanced by the larger extracellular volume⁶ and relative total sodium content⁷ of the preterm infant.

The clinical application of these studies is two-fold. First, it is apparent that the preterm newborn has lower limits of tolerance of sodium under- and overload and may respond paradoxically to an acute salt load by seeming to excrete more than is appropriate. More common is the excessive weight gain which accompanies more chronic salt loading. The second point is that extrauterine experience has not been found to alter the normal renal maturation, and sodium homeostasis seems to develop more as a function of gestational than post-natal age. □

1. Neonatal Exocrine Pancreatic Activity. *Nutrition Reviews* 31: 174-176, 1973

2. A. Aperia, O. Broberger, K. Thodenius, and R. Zetterström: Development Study of the Renal Response to an Oral Salt Load in Preterm Infants. *Acta Paediat. Scandinav.* 63: 517-524, 1974
3. A. Aperia, O. Broberger, K. Thodenius, and R. Zetterström: Renal Response to an Oral Sodium Load in Newborn Full Term Infants. *Acta Paediat. Scandinav.* 61: 670-676, 1972
4. F. L. Potter and S. T. Thierstein: Glomerular Development in the Kidney as an Index of Fetal Maturity. *J. Pediat.* 22: 695-706, 1943
5. A. Spitzer and C. M. Edelmann, Jr.: Maturational Changes in Pressure Gradients for Glomerular Filtration. *Am. J. Physiol.* 221: 1431-1435, 1971
6. G. Cassady: Bromide Space Studies in Infants of Low Birth Weight. *Pediat. Res.* 4: 14-24, 1970
7. B. M. Brenner, J. L. Troy, and T. M. Daugharty: On the Mechanism of Inhibition in Fluid Resorption by the Renal Proximal Tubule of the Volume-Expanded Rat. *J. Clin. Invest.* 50: 1596-1602, 1971

BRAIN GROWTH IN KWASHIORKOR

Clinical studies show that a reversible impairment of brain growth may occur in kwashiorkor.

Key Words: brain growth, occipito-frontal circumference, transillumination, echo encephalography, kwashiorkor, marasmic kwashiorkor

Clinical studies of cerebral function during and following recovery from infantile malnutrition are among the most important but difficult and frustrating experiments that face the nutritional investigator. Putting aside the problems of multifactorial etiology and ethical restrictions which are common to all pediatric research, the techniques of measurement of brain function are highly sophisticated and involve a number of skills including those of the neurologist, psychologist, and teacher.¹ Turning to an apparently simpler approach, that of brain growth, the investi-

gator still faces an intimidating group of problems. The difficulty of measuring the brain as opposed to some indirect assessment such as skull size, the interpretation of a reduction in size, and the use of appropriate reference standards are but three obvious and recurring points which must be considered. These are well illustrated by an interesting and detailed study performed by G. Engsner and his colleagues² working in Ethiopia. Their patients were 53 infants, between the ages of one to three years. The infants were suffering from classical kwashiorkor or marasmic-kwashiorkor³ and were admitted to the hospital for treatment. The treatment consisted of initial rehabilitation with a high-calorie and high-protein liquid diet. This was followed by

gradual weaning to a semi-solid diet and milk as clinical improvement occurred. An attempt was made to follow all the patients at monthly intervals after discharge, but for various reasons longitudinal observations were achieved in only ten cases.

Standard anthropometric information was obtained on admission to the hospital, particular care being taken in the measurement of the head circumference for which a steel tape was used. Transillumination of the skull was performed under standardized conditions as described by the same authors earlier.⁴ This was reported on a discontinuous scale of one, two, and three arbitrarily defined points. The third method of brain measurement was to perform echo-encephalography to estimate the width of the lateral ventricles. This was expressed as the ratio of the sum of the widths of the two ventricles to the diameter, presumably bi-parietal, of the head.

The head circumference measurements on admission of both boys and girls were equal to or below the 50th percentile of the standards prepared from a longitudinal study of normal Swedish infants.⁵ The authors report that the children with marasmic-kwashiorkor tended to have smaller heads than those with kwashiorkor but the difference was not significant. The test of significance used is not stated and it would be of interest to know if the results had been examined as standard deviation scores. The small head circumferences should not be accepted, however, as being necessarily pathological. It may be that Ethiopian normal standards are below those of Sweden. Having accepted the patients as abnormal by virtue of their malnutrition, it might have been more meaningful to plot the head circumference of the patients and normal Ethiopian infants⁶ against their height or height-age rather than chronological age. This would have let the authors test the possibility that head circumference and head/height ratio of malnourished children were or were not abnormal.

The heads of both types of malnourished infants showed significantly increased

transillumination in both the fronto-temporal and parieto-occipital axis. Again the statistical method used on these discontinuous results is not stated. The authors discussed the importance of scalp edema in interpreting both head circumference and transillumination measurements. While concluding that scalp edema had little effect on the head circumference they did not state if they regard it as being responsible for the increased transillumination seen at all ages studied on admission.

The lateral ventricle index was two standard deviations or more above normal in 46 out of 51 infants measured compared to the normal measurements reported by I. Sjögren.⁷ Since this index is the least likely of the three to be subject to variation in different groups of normal subjects, the findings are important.

Ten infants were followed at monthly intervals for six months. As expected there was an important increase in the weight/age ratio and related variables, such as arm circumference and triceps skinfold thickness. By contrast there was very little change in the length/age ratio over the same period, illustrating that after initial loss of edema fluid, the infants became fatter. Measurements of individual changes in height and height velocity are not reported and would have been interesting, particularly as all the measurements were made by the same investigator. Set against this background of general bodily growth the authors observed a clear cut and consistent catch-up growth of head circumference. The longitudinal study of transillumination is more difficult to interpret. Normal values change markedly with postnatal age.⁶ The ten infants were between the ages of 14 and 35 months on admission, yet their transillumination scores were averaged for the purpose of describing the changes occurring during recovery. For what they are worth the scores show a gradual normalization, which takes place, however, over a much longer time than the disappearance of the edema fluid. The mean lateral ventricle index was approximately +3 standard deviations for one month following ad-

mission. It then gradually fell to coincide with the mean for normals six months after admission. The fall in this index was the product of a more gradual increase in the external diameter of the head and a decrease in the combined width of the lateral ventricles. Again this was the most important of the longitudinal measurements as normal values do not change as a function of age between one and three years,⁷ so it was legitimate to pool the results from the patients.

The results of this study appear to demonstrate that there is a reduction in head size as a result of infantile malnutrition which is associated with an increase of skull transillumination and width of the lateral ventricles. With treatment these indices of brain size tend to return to normal, some taking as long as six months to do so. As has been pointed out above, the measurements of head circumference and transillumination are difficult to interpret for various reasons. One possibility is that the abnormal transillumination is light reflected from subarachnoid or subdural intracranial fluid. This would certainly be compatible with the increased diameter of the lateral ventricles, for it would be difficult to explain ventricular enlargement without accumulation of fluid on the surface of the brain. Extra-intracranial fluid could result from a failure of brain growth to keep pace with skull growth. A more likely explanation, however, is that the observations indicate a form of temporary communicating hydrocephalus. In developing this argument the authors point out that animal experiments have shown that hypovitaminosis A of a sufficient duration and intensity will impede the free circulation of cerebrospinal fluid, causing increased intracranial pressure.^{8,9} Vitamin A deficiency is common in Ethiopia particularly among those suffering from generalized malnutrition and this idea deserves further study. The children showed no clinical evidence of raised intracranial pressure but measurements such as blood levels of vitamin A, the clinical response of patients with kwashiorkor to vitamin A therapy for a limited period, and

the possible detection of ventricular dilatation in infants with isolated vitamin deficiency, all merit further study.

The authors' findings are corroborated to some extent by another study.¹⁰ Air encephalography was performed in 15 infants with kwashiorkor and revealed that cortical atrophy of a variable degree occurred in most cases. One year later 12 of the children had a repeat investigation and showed a complete or almost complete return to normal.

These studies do demonstrate clearly that some change does take place in the cranial contents during infantile malnutrition. Fortunately it is reversible with treatment. The nature of the change will be argued for some time. If the argument is to be constructive, however, it will provoke further research into a most important although difficult field of investigation. □

1. Nutrition, The Nervous System and Behaviour. Pan American Health Organization, Scientific Publication No. 251, 1972
2. G. Engsner, D. Habte, I. Sjögren, and B. Vahlquist: Brain Growth in Children with Kwashiorkor. A Study Using Head Circumference Measurements, Transillumination and Ultrasonic Echo Ventriculography. *Acta Paediat. Scandinav.* 63: 687-694, 1974
3. Classification of Infantile Malnutrition. *Lancet* II: 302-303, 1970
4. I. Sjögren and G. Engsner: Transillumination of the Skull in Infants and Children. Recording with a New Point Scale. *Acta Paediat. Scandinav.* 61: 426-428, 1972
5. P. Karlberg, I. Engström, H. Lichtenstein, and I. Sennberg: The Development of Children in a Swedish Urban Community. A Prospective Longitudinal Study. III. Physical Growth during the First Three Years of Life. *Acta Paediat. Scandinav.* Suppl. 187: 48-66, 1968
6. G. Engsner: Brain Growth in Privileged and Non-Privileged Ethiopian Children. A Study Using Head Circumference Measurement, Transillumination and Ultrasonic Echo Ventriculography. *J. Trop. Pediat.* (in press)
7. I. Sjögren: Echoencephalography. Evaluation Based on Records from 100 Normal Infants and Children. *Am. J. Dis. Child.* 119: 45-48, 1970

8. H. D. Eaton: Chronic Bovine Hypo- and Hypervitaminosis A and Cerebrospinal Fluid Pressure. *Am. J. Clin. Nutrition* 22: 1070-1080, 1969
9. K. C. Hayes, H. L. McCombs, and T. P. Faherty: The Fine Structure of Vitamin A Deficiency. II. Arachnoid Granulations and CSF Pressure. *Brain* 94: 213-224, 1971
10. E. Marcondes, A. B. Lefèvre, D. V. M. Machado, S. Gazal, A. Cavallo, A. Spina-França, B. W. I. Ferreira, C. Lamego, D. Barbieri, G. Quarentei, L. P. Vallada, M. C. M. Briquet, M. I. Valente, N. G. Barros, N. Setian, and T. Stangherlin: Desenvolvimento neuropsicomotor da criança desnutrida. I. Má nutrição protéica. *Rev. Brasil Psiquiat.* 3: 173, 1969

LYMPHOCYTE FUNCTION IN MALNUTRITION

New work on in vitro and in vivo tests of lymphocyte function in malnourished children and small-for-dates babies are reviewed.

Key Words: malnutrition, lymphocyte, cell-mediated immunity, rosette-forming cells

A recent review¹ described new work on the immunocompetence of adults suffering from malnutrition. A study by R. K. Chandra² extended this to a younger age group. Three tests of the immune response were measured in each of 15 children diagnosed as being malnourished. The criteria of malnourishment were failure to thrive, poor dietary history, the usual evidence of fat and hair loss, and a weight of less than 80 percent of the 50th percentile for their age. Ten healthy children were used as controls.

Lymphocytes forming the center of spontaneous rosettes of sheep red cells are generally considered to belong to the category of thymus-dependent or T-cells.³ In the malnourished children there was a statistically significant decrease in the percent of the total lymphocyte population possessing this characteristic, compared with those from normal children. Thus, the initial mean percent for the malnourished group was 23 percent, rising to 60 percent after dietary treatment. The percent of T-lymphocytes in the control group was 71 percent. Since Chandra previously demonstrated involution of lymphoid tissue including the thymus⁴ and the occurrence of lymphopenia in the blood in at least some

cases,⁵ the absolute number of T-lymphocytes may in fact be even relatively lower in the malnourished child.

Other lymphocyte functions were also measured. The plant lectin, phytohemagglutinin, activates dormant small lymphocytes to enter the cell cycle, replicate their DNA, and undergo mitosis. The capacity to do so is also generally considered to be a property of that fraction of lymphocytes known as T-cells.⁶ The T-cells act as the memory cells for a particular antigen as opposed to the B-cells or bone-marrow derived lymphocytes which can synthesize the antibody to that antigen. Stimulation induced by phytohemagglutinin is measured by the incorporation of radioactive thymidine into DNA, and was shown to be low (on the average of 35 times greater than that of unstimulated cultures) in malnourished children on presentation when compared to their level after dietary restitution (97 times control) or to the group of normal children (111 times control). Of course these lower figures may be due in part to an absolute reduction in the number of lymphocytes capable of responding as well as to a possible qualitative functional defect. Data as to absolute values for peripheral blood lymphocytes would therefore have been of interest in these children but these are not reported.

The third estimate of T-cell function measured was the ability to confer sensitivity to an antigen to which there had been no previous exposure. Dinitrochlorobenzene (DCNB) was applied to the skin and a delayed sensitivity reaction looked for after the injection of the chemical into another cutaneous site one month later. Only two of the 15 malnourished children gave a positive response. In contradistinction such a result was obtained in all of the ten healthy children.

A similar project was undertaken by A. C. Ferguson and his co-workers⁷ studying a group of malnourished children in Ghana. In a group of ten, three were marasmic and seven had kwashiorkor. In vivo cell-mediated immunity was assessed by delayed hypersensitivity skin testing using PHA, monilia, streptokinase/streptodornase, keyhole limpet hemocyanin, and purified protein derivative as antigens. When compared to ten normally nourished children, positive responses were obtained on notably fewer occasions, e.g. for monilia two out of ten for the malnourished group, compared with eight out of ten for the control group.

In vitro cellular immunity was also assessed. In this study there was no significant difference in the total lymphocyte count in either group. In vitro stimulation of lymphocytes with PHA showed similarly no significant difference between the two groups as a whole although three malnourished children had rather low results. The numbers of rosette-forming, or T-lymphocytes did, however, show a distinct difference. An average result of 16.6 percent was obtained for the malnourished group and 59.7 percent for the control group. Therefore, these results confirm those of Chandra using the same test. Also in this second study, rosette-forming cells returned to a normal percent after one to three weeks on a high protein, high calorie diet.

These studies were also extended to include the assessment of rosette-forming cells in ten small-for-dates babies as compared with ten infants with normal birth weights. There was a significant difference

in the percent of such cells, 49.2 percent, when compared to the controls, 65.1 percent. The authors suggest this may explain in part the increased susceptibility of such small-for-dates infants to infection and ties up with the reported hypoplasia of their thymic tissue.

These studies therefore go some way in suggesting at least part of the possible pathogenesis of the known increased susceptibility to infection of malnourished individuals. The possible basis of the T-cell defect remains a matter for speculation. It might be lack of a single or small group of key nutrients, secondary to the elevated cortisol levels known to occur in such individuals⁸ or a depression of some as yet unknown thymic hormone. Whatever the basis, it is remarkable and fortunate that such changes are rapidly reversible with attainment of good nutrition. □

1. Immunocompetence in Adult Malnutrition. *Nutrition Reviews* 32: 201-202, 1974
2. R. K. Chandra: Rosette-forming T Lymphocytes and Cell-Mediated Immunity in Malnutrition. *Brit. Med. J.* 3: 608-609, 1974
3. C. G. Craddock, R. Longmire, and R. McMillan in *Ontogeny of Acquired Immunity*. Pp. 113 Ciba Foundation Symposium, Amsterdam, 1971
4. P. M. Smythe, M. Schonland, G. G. Brereton-Stiles, H. M. Coovadia, H. J. Grace, W. E. K. Loening, A. Mafoyané, M. A. Parent, and G. H. Vos: Thymolymphatic Deficiency and Depression of Cell-Mediated Immunity in Protein-Calorie Malnutrition. *Lancet* II: 939-944, 1971
5. R. K. Chandra: Immunocompetence in Undernutrition. *J. Pediat.* 81: 1194-1200, 1972
6. D. Catovsky, J. M. Goldman, A. Okos, B. Frisch, and D. A. Galton: T-Lymphoblastic Leukaemia: A Distinct Variant of Acute Leukaemia. *Brit. Med. J.* 2: 643-646, 1974
7. A. C. Ferguson, G. J. Lawlor, Jr., C. G. Neumann, W. Oh, and E. R. Stiehm: Decreased Rosette-Forming Lymphocytes in Malnutrition and Intrauterine Growth Retardation. *J. Pediat.* 85: 717-723, 1974
8. S. L. Naeye, M. M. Diener, H. T. Harke, Jr., and W. A. Blanc: Relation of Poverty and Race to Birth Weight and Organ and Cell Structure in the Newborn. *Pediat. Res.* 5: 17-22, 1971

PHILOSOPHICAL
TRANSACTIONS
OF THE
ROYAL SOCIETY
OF
LONDON.

FOR THE YEAR MDCCCXXVII.

PART I.

Volume CXVII:355-388, 1827

XXIII. *On the ultimate composition of simple alimentary substances; with some preliminary remarks on the analysis of organized bodies in general.*
By WILLIAM PROUT, M.D. F.R.S.

Read June 14, 1827.

THE present being the first of several communications on the same subject which I hope to have the honour of laying before the Royal Society, a few observations on the origin and object of the whole series may not be deemed irrelevant.

• • •

Organic chemistry is confessedly one of the most difficult departments of the science; and though much has been done, and more attempted on the subject, it is yet in a very imperfect and unsatisfactory state; and it must be frankly admitted that Physiology and Pathology have derived less advantage from this most promising and really powerful of the auxiliary sciences, than might have been expected. To explain this perhaps would not be difficult; but as the explanation would be misplaced here, I shall merely observe, that

23 JUL 1975

dissatisfied with the old modes of inquiry, I determined to attempt a different one, and keeping in view the notions I had originally formed respecting chemical combinations, proposed to myself to investigate the modes in which the three or four elementary substances entering into the composition of organized bodies are associated, so as to constitute the infinite variety occurring in nature.

With these views my first object was to determine the exact composition of the most simple and best defined organic compounds, such as sugar, and the vegetable acids, a point that had been several times before attempted, but, as it appeared to me, without complete success. About the same time also albumen and other animal products, as urea, lithic acid, &c. were examined with similar views. The subject of digestion, however, had for a long time occupied my particular attention; and by degrees I had come to the conclusion, that the principal alimentary matters employed by man, and the more perfect animals, might be reduced to three great classes, namely, the *saccharine*, the *oily*, and the *albuminous*: hence, it was determined to investigate these in the first place, and their exact composition being ascertained, to inquire afterwards into the changes induced in them by the action of the stomach and other organs during the subsequent processes of assimilation. In conformity with this plan, the object of the present communication is the consideration of the first class or family above-mentioned, namely, the *saccharine*.

Preliminary observations on the analysis of organised substances.

Vegetable substances contain at least two elements, hydrogen and carbon; and most generally three, hydrogen, carbon, and oxygen. Animal substances are still more complicated; and besides the above three, usually involve a fourth element, namely, azote, to which they appear to owe many of their peculiar properties.

• • •

Of the Saccharine principle.

	Carbon.	Water.
Pure sugar candy	42.85	57.15
* Impure sugar candy	41.5 to 42.5	58.5 to 57.5
East India sugar candy (v) .	41.9	58.1
English refined sugar.	41.5 to 42.5	58.5 to 57.5
East India refined sugar (v).	42.2	57.8
Maple sugar (v)	42.1	57.9
Beet-root sugar (v)	42.1	57.9
East India moist sugar (v). .	40.88	59.12
Sugar of diabetic urine . . .	36 to 40?	64 to 60?
Sugar of Narbonne honey .	36.36	63.63
Sugar from starch.	36.2	63.8

• • •

In conclusion, I wish to observe, that I purposely abstain at present from making any further observations on the preceding results than those already given. I do this for several reasons: in the first place, such observations will appear with greater effect, when the whole of the facts in my possession are laid before the public; and secondly, I consider that data which lead to such important conclusions as these appear to do, cannot be too firmly established; I therefore, in the mean time, earnestly invite chemists in general to repeat them, and thus either to confirm them, or point out their errors; and for the sake of those who may be inclined to take this trouble, I shall close this part of the subject with the following remarks: 1. The multiples of hydrogen, carbon, and oxygen, are assumed in the preceding calculations as, 1 : 6 : 8. 2. The results given are, on all essential points, the means of many experiments, the differences among which are either inappreciable, or at most vary from .01 to .03 of a cubic inch in from 5 to 8 cubic inches of carbonic acid or oxygen gas; the greatest differences in general, being for obvious reasons, found among merorganized bodies; and hence the analyses of these are usually stated to the first decimal figure only. 3. As rules to be observed, I would say, that a single result should never be registered, nor a single calculation made, till the operator has made himself complete master of his apparatus, and carefully studied the nature of the substance to be analyzed; for different substances often require very different management: that two or three results should never be relied on; the minute quantities here sought can be only obtained, like those of astronomy, by repeated observations: and lastly, the utmost care should be taken that the substances operated on be *pure*, a point of greater importance, and frequently of more difficult accomplishment than any other, and one that has caused me more trouble than all the rest put together.

LENGTHS OF POLY- γ -GLUTAMYL CHAINS IN NATURAL FOLATES

An assessment of the peptide chain lengths of natural folates has been accomplished by reductive cleavage of the pteridine portion from the series of p-aminobenzoylglutamyl poly- γ -glutamates, the latter being separated by chromatography. Folates with four glutamate residues predominate in certain folate-requiring bacteria. Uptake of folic acid into hepatic folates in the rat is rapid with the early appearance of tetra- and pentaglutamate forms. After 24 hours, over 90 percent were in the penta- and hexaglutamyl forms, with the remainder consisting of tetra- and heptaglutamates.

Key Words: folates, poly- γ -glutamates

There are numerous natural folates that result from variations in substituents on the pteroyl moiety, as well as in the chain length of γ -glutamyl residues.¹ Present evidence indicates that it is actually the poly- γ -glutamates of folic acid that function in the coenzymatic roles.²⁻⁵ Most estimations of the quantities of the folates with different chain lengths have relied on separations by chromatographic means and differential microbiological assays before and after deconjugation with pteroylglutamyl- γ -polyglutamyl hydrolases.⁶⁻¹⁰ However, the failure of some of the pteroyl-substituted forms to separate readily from the mono- to polyglutamate derivatives of other forms and the difficulties inherent in assays with overlapping specificities have led to a limitation in the applicability of earlier methodologies and to some question as to the validity of the quantitations achieved.

C. M. Baugh and his co-workers¹¹ developed a method which avoids the earlier difficulties in assessing the lengths of poly- γ -glutamate chains present in natural folates without regard for the presence or absence of alterations at nitrogen 5 or 10 in the pteroyl portion of such compounds. This method involves removal of the pteridine portion by reductive cleavage at the

C(9)-N(10) bond with Zn/HCl. The homogeneous series of p-aminobenzoylglutamyl polyglutamates and the corresponding N(10)-methyl compounds, which result when a substituent (methyl, methylene, or methenyl) is present in the natural folates, can then be separated cleanly in sequence by elution from a column of diethylaminoethyl-cellulose (chloride) with a linear gradient of buffered sodium chloride. The volume of salt solution required to elute each derivative, which contained 1 to 7 glutamate residues, under the defined conditions was determined with ¹⁴C-labeled and nonradioactive compounds that had been synthesized. The amount of each compound was assessed by ultraviolet absorbance contributed by the (N-methyl) p-aminobenzoyl moiety and by the radioactivity contained within the uniformly labeled glutamate portion.

The distributions of poly- γ -glutamyl chain lengths in bacterial folates were determined for three folate-dependent strains.¹¹ The respective Difco folate-free assay media were used to culture *Lactobacillus casei* 7496, *Streptococcus faecium* 8043, and a methotrexate-resistant strain of the latter. Liquid media contained 0.8 μ Moles of pteroyl[U-¹⁴C]glutamic acid per liter with the addition of 5 mg of methotrexate in the case of the resistant strain. Incubations were at 37° for 24

hours, after which cells were collected by centrifugation and washed twice with isotonic saline. Folates were extracted by homogenizing the cells in 6 M urea made 5 percent in trichloroacetic acid. Protein was removed by centrifugation and the supernatant adjusted to pH 6 to 7 and diluted five-fold before pouring onto a DEAE-cellulose (chloride) column. This column was washed with water and stripped with 0.5 N HCl. Tubes containing radioactivity were pooled and the radioactive folates subjected to reductive cleavage. Recovery of radioactivity was found to be consistently 85 to 90 percent. The resulting [^{14}C]p-aminobenzoylglutamates were fractionated by further chromatography on columns of DEAE-cellulose as had been shown to separate the synthetic compounds.

The percent of folates with number of glutamate residues (in parentheses) found for *L. casei* were: 3.2 (1), 0 (2), 9.3 (3), 59.4 (4), 23 (5), 5.1 (6), and 0 (7). For *S. faecium*, values determined were: 16.5 (1), 8.5 (2), 20 (3), 54.5 (4), and 0 for (5), (6), and (7). Values for the methotrexate-resistant strain of *S. faecium* were: 1 (1), 5.7 (2), 6.9 (3), 81.3 (4), 5.1 (5), and 0 for (6) and (7). An interesting consequence of these results is the correction of previous estimates made by others who used less reliable methods. The earlier claim⁷ that all folates of *L. casei* possess more than six glutamyl residues is incompatible with the present finding¹¹ that about 60 percent of the total folate was in the tetraglutamate form, while only 5 percent of the hexa- and none of the heptaglutamate derivatives were found. Even more disparate is a report⁶ that 75 percent of the folates of *L. casei* have more than seven glutamyl residues and that 24 percent of the pentaglutamate form is present in *S. faecium*.

Moreover, this work of Baugh et al.¹¹ lends some insight into the cause for the resistance of the one strain of *S. faecium* to methotrexate. In addition to studying the chain length of folates present, these investigators noted that no radioactivity from methotrexate labeled with

[U- ^{14}C] glutamate was incorporated into the growing cells. It is probable that the resistance in this mutant is conferred by a transport alteration which excludes the folate antagonist.

In a companion paper,¹² G. I. Leslie and C. M. Baugh report on their study of the uptake of pteroyl[^{14}C] glutamate into rat liver and the subsequent conversion of the monoglutamate to polyglutamate forms in that organ. Male Sprague-Dawley rats, weighing between 200 and 250 g and maintained on a nutritionally complete diet, were injected intraperitoneally with 175 μg of [^{14}C] folate, which had been synthesized by a solid phase method.¹³ The animals were sacrificed at fixed intervals and livers extirpated and homogenized for assay generally as was done with bacteria.

Maximal uptake of the labeled folate occurred after 6 hours when 4 to 4.5 percent of the radioactivity injected was found in the liver. This level of radioactivity decreased past 24 hours until 2.9 percent was present after a week and only 0.5 percent after a month. The distribution of folate types with respect to length of the γ -glutamyl chain was found to change from a preponderance of the [^{14}C] monoglutamate form within an hour after injection to an increasing amount of the tetra- and then pentaglutamate up to a day. By a week, the hexaglutamate (57 percent) was the main form present, though significant amounts (36 percent) of the penta- were still found. As the amounts of [^{14}C] heptaglutamate (5.2 to 5.4 percent) were relatively constant between the first and second week following injection, and the amounts of other labeled forms generated from the initial [^{14}C] monoglutamate were generally similar after a week, it appears that an injected dose of the monoglutamate reaches an equilibrium with the hepatic folate pools by this time. By 28 days, a larger fraction of the ^{14}C -label (26.2 percent) was found in the heptaglutamate, while over half of the label (53.6 percent) was still present as hexaglutamate, and only about a fifth (20.2 percent) remained as the pentaglutamate.

The results reported by Leslie and Baugh^{1,2} appear more reliable than earlier ones and surely indicate the principal trends in the in vivo conversion of pteroylglutamic acid to the poly- γ -glutamate forms that predominate within cells utilizing these coenzyme forms for one-carbon metabolism. However, the use of only one type of label in a specific portion of the single dose of folic acid injected should be viewed as incomplete information. Non-labeled forms that may have half-lives of far longer duration than the time-course chosen, or any forms that are not in equilibrium with the circulating folates, would not have been detected. The investigations of Baugh and his co-workers, though, have already advanced our understanding of the distribution and interconversions of natural folates. \square

1. T. Shiota: The Biosynthesis of Folic Acid and 6-Substituted Pteridine Derivatives. *Comp. Biochem.* 21: 111-152, 1970
2. P. J. Large and J. R. Quayle: Microbial Growth on C₁ Compounds. 5. Enzyme Activities in Extracts of *Pseudomonas* AM1. *Biochem. J.* 87: 386-396, 1963
3. J. R. Guest and K. M. Jones: Tetrahydropteroyltriglutamate as a Cofactor of Methionine Synthesis. *Biochem. J.* 75: 12P-13P, 1960
4. C. D. Whitfield and H. Weissbach: Binding of Substrate to N⁵-Methyl-Tetrahydropteroyltriglutamate-Homocysteine Transmethylase. *Biochem. Biophys. Res. Commun.* 33: 996-1003, 1968
5. E. Burton, J. Selhub, and W. Sakami: The Substrate Specificity of 5-Methyltetrahydropteroyltriglutamate-Homocysteine Methyltransferase. *Biochem. J.* 111: 793-795, 1969
6. K. U. Buehring, T. Tamura, and E. L. R. Stokstad: Folate Coenzymes of *Lactobacillus casei* and *Streptococcus faecalis*. *J. Biol. Chem.* 249: 1081-1089, 1974
7. Y. S. Shin, K. U. Buehring, and E. L. R. Stokstad: Separation of Folic Acid Compounds by Gel Chromatography on Sephadex G-15 and G-25. *J. Biol. Chem.* 247: 7266-7269, 1972
8. Y. S. Shin, M. A. Williams, and E. L. R. Stokstad: Identification of Folic Acid Compounds in Rat Liver. *Biochem. Biophys. Res. Commun.* 47: 35-43, 1972
9. W. W. Osborne-White and R. M. Smith: Identification and Measurement of the Folates in Sheep Liver. *Biochem. J.* 136: 265-278, 1973
10. R. Corrocher, B. K. Bhuyan, and A. V. Hoffbrand: Composition of Pteroylpolyglutamates (Conjugated Folates) in Guinea-Pig Liver and their Formation From Folic Acid. *Clin. Sci.* 43: 799-813, 1972
11. C. M. Baugh, E. Braverman, and M. G. Nair: The Identification of Poly- γ -Glutamyl Chain Lengths in Bacterial Folates. *Biochemistry* 13: 4952-4957, 1974
12. G. I. Leslie and C. M. Baugh: The Uptake of Pteroyl[¹⁴C]Glutamic Acid into Rat Liver and Its Incorporation into the Natural Pteroyl Poly- γ -Glutamates of that Organ. *Biochemistry* 13: 4957-4961, 1974
13. C. L. Krumdieck and C. M. Baugh: The Solid-Phase Synthesis of Polyglutamates of Folic Acid. *Biochemistry* 8: 1568-1572, 1969

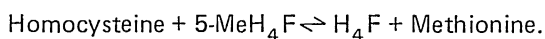
FOLIC ACID METABOLISM IN VITAMIN B₁₂ DEFICIENCY

Vitamin B₁₂ deficiency leads to secondary folate deficiency. Methionine alleviates it. Operation of the "methyl trap" due to a block in the transmethylation reaction, a reduced uptake of folate by the cells, and diminished synthesis of folate polyglutamates are the alternative theories advanced to explain this condition.

Key Words: folate, vitamin B₁₂, methionine, methyl trap, folate polyglutamates, folate monoglutamates, S-adenosyl methionine, transmethylation

Vitamin B₁₂ folate deficiency produces a secondary folate deficiency in man and animals.¹ Thus in B₁₂ deficiency, urinary excretion of formiminoglutamate (FIGLU) and aminoimidazole carboxamide are elevated.

An important locus of interaction between these two vitamins is the enzyme 5-MeH₄F-homocysteine transmethylation which catalyzes the synthesis of methionine from homocysteine.



According to the methyl trap hypothesis which has been advanced to explain secondary folate deficiency associated with primary B₁₂ deficiency,¹ in B₁₂ deficiency, folate gets blocked as 5-MeH₄F due to an impaired transmethylation reaction and an essentially irreversible methylene H₄F reductase reaction.

Elevated levels of serum folate have been reported in some patients with pernicious anemia and in B₁₂-deficient animals. Since the major folacin compound in circulation in these situations is 5-MeH₄F, this observation is quoted to support the methyl trap theory. However, the data on 5-MeH₄F levels in tissues of vitamin B₁₂-deficient animals and plasma of patients with pernicious anemia are equivocal. Therefore, this theory cannot satisfactorily explain the depletion of tissue folates frequently observed in B₁₂-deficient animals. Also, plasma folate in rats is less than 1 percent of the folate contained in the liver

and may not be representative of other tissues.

Methionine administration appears to alleviate the folate deficiency secondary to vitamin B₁₂ deficiency in animals. The basis for the methionine effect is not known but S-adenosyl methionine inhibits methylene H₄F reductase in vitro. Such an inhibition in vivo would divert folate from accumulation in its methyl tetrahydrofolate form.

S. W. Thenen and E. L. R. Stokstad² found that monoglutamate forms of folate are not affected in the livers of B₁₂-deficient rats, but polyglutamates (methyl and non-methyl forms) show a fall which can be corrected with methionine as well as with vitamin B₁₂. The ratio of methyl pentaglutamate to total non-methylated reduced pentaglutamate was markedly elevated in the livers of B₁₂-deficient rats. This was returned to normal by the administration of vitamin B₁₂ or methionine. They interpret this as evidence in support of the methyl trap theory and conclude that dietary cobalamine and methionine regulate the concentration of folate polyglutamates but not the monoglutamates. This does not, however, explain the fall in total polyglutamates, including the methyl form, in B₁₂ deficiency and their restoration after supplementation.

In a more recent paper, A. J. Vidal and E. L. R. Stokstad³ examined the urinary excretion of folate derivatives and the concentration of liver folates after a single tracer dose of tritiated pteroyl glutamate in B₁₂-deficient rats with or without administration of methionine (0.2 percent in diet-

ary protein supplement) or its analogue ethionine. In the same study, the effect of methionine and/or vitamin B₁₂ on liver S-adenosyl methionine levels was also investigated.

Liver uptake of folate as judged by concentration of the label was lower in the B₁₂-deficient animals. Urinary excretion was higher, 65 percent being 5-CH₃H₄F. Vitamin B₁₂, methionine, and/or ethionine reduced the urinary folate and the percent of the total present as the methyl derivative (45 percent).

Hepatic S-adenosyl methionine was low in the deficient group but returned to normal after treatment with vitamin B₁₂ (100 µg per kilogram of diet) and/or methionine (15 g per kilogram of diet). Based on these data the authors conclude that through the operation of the methyl trap, the uptake of folate by the liver and the synthesis of polyglutamates is impaired. The unutilized 5-CH₃H₄F is excreted in the urine. From the results on changes in liver S-adenosyl methionine the authors suggest that there is feedback inhibition of methylene tetrahydrofolate reductase by the active form of methionine *in vivo*.

Monoglutamates, mainly 5-CH₃H₄F are the only transport form in the body fluids. On the other hand polyglutamates are probably the coenzyme forms inside the cells. In some systems only polyglutamates show activity and polyglutamate coenzymes have a higher affinity for the enzymes. I. Chanarin and co-workers, who strongly oppose the methyl trap theory recently showed diminished polyglutamates but not monoglutamates in erythrocytes of patients with pernicious anemia.⁴ This group feels that the primary block in vitamin B₁₂ deficiency lies in the conversion of monoglutamates to polyglutamates.

Defective transport of 5-CH₃H₄F into erythrocytes of patients of pernicious anemia has been reported, but Chanarin et al⁴ are of the opinion that this is secondary to the failure of polyglutamate synthesis inside the cells. Furthermore, these workers comment that since the primary clinical lesions of B₁₂ deficiency in man and ani-

mals are not similar, conclusions based on animal studies may not always be valid.

R. M. Smith and co-workers in Australia studied the B₁₂-folate-methionine interaction in B₁₂-deficient sheep.^{5,6} Although B₁₂-depleted sheep contained significantly lower concentration of non-methylated folates in their livers than B₁₂-supplemented animals, there was a uniform depletion of both conjugated and unconjugated forms within these classes. There was also a small but uniform restoration of both conjugated as well as the unconjugated forms on treatment with physiological amounts of methionine. Since both methylated and the non-methylated forms were reduced in B₁₂ deficiency, the authors question the methyl trap theory, although they comment that a higher concentration of methylated form may be due to block in methyl transferase reaction. Their data fail to support the polyglutamate synthesis theory.

Since ATP facilitates polyglutamate synthesis, and nicotinamide coenzymes are essential for interconversion of folate vitamers, the levels of these as well as K⁺/Na⁺ ratios in freeze clamped sheep livers were examined.⁵ Levels of NAD⁺, NADH, NADPH, and ATP were reduced and the K⁺/Na⁺ ratio elevated in B₁₂ deficiency.⁵

A study of liver enzymes⁶ showed that the activities of dihydrofolate reductase and 5-CH₃H₄F-homocysteine trans-methylase were significantly diminished in the livers of B₁₂-deficient sheep and were not altered by methionine supplementation.⁶ While the methylene tetrahydrofolate reductase was inhibited by S-adenosyl methionine *in vitro* it was not inhibited by levels of this metabolite found in the liver after the administration of physiological amounts of methionine. Pteroyl polyglutamate synthetase activity was significantly higher in the livers of deficient animals and fell in methionine supplemented animals. The authors feel that the rise in polyglutamate synthetase may be an attempt to conserve folates.

To test folate transport in B₁₂ deficiency, the effect of intravenous administration of methionine on the transport of

methotrexate in liver slices was studied.⁶ Methotrexate shares the folate transport system in mammals and unidirectional transport can be studied, since it is not further metabolized. Methionine treatment was found to increase methotrexate uptake suggesting that it also probably promotes the uptake of natural folates from plasma into the liver. The authors feel that B₁₂ may help folate transport into the cell by increasing the intracellular concentration of methionine through the transmethylase system.

Thus, operation of methyl trap, as well as diminished folate transport and lowered polyglutamate synthesis have been observed in B₁₂-deficient animals and/or man. The primary lesion remains a moot point. □

1. R. L. Blakley. *The Bio-Chemistry of Folic Acid and Related Pteridines*. Pp. 453-460. North - Holland Publishing Co. Amsterdam-London, 1969

2. S. W. Thenen and E. L. R. Stokstad: Effect of Methionine on Specific Folate Coenzyme Pools in Vitamin B₁₂ Deficient and Supplemented Rats. *J. Nutrition* 103: 363-370, 1973
3. A. J. Vidal and E. L. R. Stokstad: Urinary Excretion of 5-Methyltetrahydrofolate and Liver S-Adenosylmethionine Levels of Rats Fed a Vitamin B₁₂-Deficient Diet. *Biochem. Biophys. Acta*. 362: 245-257, 1974
4. I. Chanarin, J. Perry, and M. Lumb: The Biochemical Lesion in Vitamin B₁₂ Deficiency in Man. *Lancet* 1: 1251-1252, 1974
5. R. M. Smith, W. S. Osborne-White, and J. M. Gawthorne: Folic Acid Metabolism in Vitamin B₁₂ Deficient Sheep. Effects of Injected Methionine on Liver Constituents Associated with Folate Metabolism. *Biochem. J.* 142: 105-117, 1974
6. J. M. Gawthorne and R. M. Smith: Folic Acid Metabolism in Vitamin B₁₂ Deficient Sheep. Effects of Injected Methionine on Methotrexate Transport and the Activity of Enzymes Associated with Folate Metabolism in Liver. *Biochem. J.* 142: 119-126, 1974

INTERCONVERSION OF BACTERIAL AGENTS CAUSING BOTULISM AND GAS GANGRENE

Experiments have demonstrated the necessity of bacteriophage in Clostridium botulinum for neurotoxin production. Using specific toxin-inducing phages, a non-toxicogenic C. botulinum type C organism was converted to toxicogenic C. novyi type A as well as C. botulinum type C or D.

Key Words: *Clostridium*, botulinum, toxin, inter-conversion, bacteriophage

Of the various toxic or infectious disease agents that may be transmitted by food, botulinum toxins are perhaps the most dreaded. This fact is due to their extremely high potency for many animal species including man. C. Lamanna,¹ who was one of the first people to obtain botulinum toxin in the crystalline form, described this exotoxin as the "most poisonous poison."

The bacterial agent, *Clostridium botulinum*, is one species among several gram-positive, anaerobic, spore-forming bacilli capable of producing a variety of potent toxin proteins. Seven immunologically dis-

tinct neurotoxins (A through G) have been obtained from *C. botulinum*, although most type-identified human cases in the United States have been traced to types A, B, and E. The case fatality rate has been high (>60 percent) in the past but has shown a decline in the last three decades.²

Clostridium tetani, another species, produces the well-known causative agent of tetanus. Spores of the organism in soil or manure may contaminate wounds and upon germination establish a site of infection from which a potent neurotoxin is disseminated to the nerve tissues. Other species of *Clostridium* are notorious for their ability to proliferate in traumatized tissues and cause extensive necrosis referred

to as gangrene. *Clostridium perfringens*, *C. sporogenes*, and *C. novyi* are examples of this category. These species are characterized by their ability to form tissue-destroying enzymes such as immunologically distinct lecithinases, collagenases, and other biologically active substances. *Clostridium perfringens* type A in the sporulating phase synthesizes a potent enterotoxin which is the principal agent responsible for a fairly common food poisoning in humans. The toxin is a protein (molecular weight of about 36,000) that exerts a profound effect on the secretory activity and contractility of the small intestine.³

The seven immunological types of *C. botulinum* have been divided into four categories based on common biochemical and serological properties.⁴ One of these, Group III, includes *C. novyi* type A as well as *C. botulinum* types C and D. Toxicogenic strains of *C. botulinum* C and D and *C. novyi* A are readily differentiated, however, on the basis of distinct differences in their respective major toxins. The botulinum toxins are paralytic poisons which inhibit the release of acetylcholine at cholinergic nerve junctions or terminals, whereas type A *C. novyi* produces a lethal alpha toxin not formed by *C. botulinum*.

It has been shown that conversion of non-toxicogenic strains of *C. botulinum* C and D to respective toxin producers requires the presence of type-specific bacteriophages and that prophages must persist in the bacterial cells for continued toxigenicity.⁵ A recent study of unusual and far-reaching importance has extended these findings and demonstrated not only the interconversion of types C and D *C. botulinum* but also the conversion of a non-toxicogenic type C *C. botulinum* to a toxicogenic *C. novyi* type A.⁶ These conversions were effected through the use of specific bacteriophages in each case. The series of experiments that established these phenomena are outlined below.

Starting with a prophage-bearing toxicogenic strain of type C *C. botulinum* (#162) a non-toxicogenic isolate (HS37) was obtained through the process of heat-

treatment of spores of #162 followed by plating and selection of colonies that were devoid of prophage and toxigenicity. This non-toxicogenic strain, HS37, was susceptible to reinfection with type C toxin-inducing phage which re-established the type C toxicogenic strain. It was also found, however, that HS37 could be infected with a type D toxin-inducing strain of phage and in this way type D *C. botulinum* toxicogenic organisms could be derived. More significantly, HS37 was also susceptible to phages obtained from *C. novyi* type A. Infection of HS37 with a strain of phage purified from a type A alpha toxin producing strain of *C. novyi* resulted in formation of toxicogenic *C. novyi* type A from the original *C. botulinum* organism. Thus, three immunologically distinct toxins could be produced by a common bacterial strain (HS37) following infection with the respective toxin-inducing bacteriophages. It was also shown that infection of HS37 with a phage derived from a non-toxicogenic strain of *C. novyi* failed to induce toxigenicity in HS37.

The necessity of continued presence of phage for toxigenicity was also demonstrated. Heating the spores from each derived toxicogenic strain at 70°C for 15 minutes resulted in inactivation of prophage from a portion of the population and a simultaneous loss of toxigenicity in the respective progeny. Other isolates from this treatment which retained their phage component (a majority) also remained toxigenic. The derived non-toxicogenic isolates were susceptible to toxin-inducing phages from *C. botulinum* types C or D and *C. novyi* type A, each of which again induced its specific type of toxigenicity. The toxicogenic survivors from the heat treatment were immune to homologous phage. When strain HS37 was infected with toxin-inducing phages of *C. botulinum* type C or D, respectively, it became resistant to infection with both types C and D phages but continued to be sensitive to the toxin-inducing phage from *C. novyi*. Conversely, HS37 infected with type A *C. novyi* phage remained sensitive to phages C and D of *C. botulinum*. The authors did not indicate

what characteristics were present in strains bearing dual phage infections.

Further work is required to determine if other isolates of *C. novyi* containing prophage can be freed of its viral infection and then converted to *C. botulinum* type C or D employing specific bacteriophages.

This important study undoubtedly will open new areas of research on the mechanism of toxin production by the Clostridia. Also, the possibility that a single strain of bacteria could be responsible under certain conditions for both botulism and gas gangrene must certainly be considered. □

1. C. Lamanna: The Most Poisonous Poison. *Science* 130: 763-772, 1959
2. Center for Disease Control: Botulism in the United States, 1899-1973. Handbook for

Epidemiologists, Clinicians, and Laboratory Workers, p. 2, 1974

3. A.H.W. Hauschild, R. Hilsheimer, and W. G. Martin: Improved Purification and Further Characterization of *Clostridium perfringens* Type A Enterotoxin. *Canad. J. Microbiol.* 19: 1379-1382, 1973
4. Proceedings of the First U.S.-Japan Conference on Toxic Microorganisms. Published by the UJNR Joint Panels on Toxic Microorganisms and the U.S. Department of the Interior, p. 228
5. M. W. Eklund and F. T. Poysky: Interconversion of Type C and D Strains of *Clostridium botulinum* by Specific Bacteriophages. *Applied Microbiol.* 27: 251-258, 1974
6. M. W. Eklund, F. T. Poysky, J. A. Meyers, and G. A. Pelroy: Interspecies Conversion of *Clostridium botulinum* Type C to *Clostridium novyi* Type A by Bacteriophage. *Science* 186: 456-458, 1974

New

Unique

INDEX OF NUTRITION EDUCATION MATERIALS

The Index of Nutrition Education Materials has been prepared by the Office of Education of the Nutrition Foundation to assist teachers and the general public in acquiring useful teaching aids and educational materials.

The Index lists more than 1,000 booklets, pamphlets, audio-visual aids and lists of recommended books available from federal agencies, professional societies, and educational organizations or foundations. Other sources for education material are included.

Gives addresses of important local contacts and sources in each state.

Source, subject matter, readership level and description are given for each publication in color-coded sections for easy reference. \$5.00 postpaid

Order from:
OFFICE OF EDUCATION
THE NUTRITION FOUNDATION
888 Seventeenth Street, N.W.
Washington, D. C. 20006

THE EFFECTS OF FOOD PROCESSING ON NUTRITIONAL VALUES

*A Scientific Status Summary by the INSTITUTE OF FOOD TECHNOLOGISTS'
EXPERT PANEL ON FOOD SAFETY AND NUTRITION & THE COMMITTEE ON
PUBLIC INFORMATION*

The major goal of food preservation is to free man from total dependence on geography and climate in providing for his nutritional needs and wants. While there are a few areas of the world where fresh fruits and vegetables are available year-round, without food preservation methods most of the world's population would be faced with a "feast or famine" situation—a large volume, large selection during a short harvest period, followed by a long winter and spring, eating only a limited number of staples such as the grains and a few root crops.

While all preservation methods contribute to this major benefit, each also operates in a trade-off situation—they lead to an inevitable loss in certain nutrients. Nutritional losses occur whether food is processed commercially or at home, and they also occur if a food is stored in an unprocessed state.

The major consideration, then, in evaluating food processing from a nutritional standpoint is the trade-off between increased food availability and the effects each of the various kinds of processing have on nutrition (including the effects of no processing at all). Also to be considered are the *degree* or *extent* of loss (it is often greater in home processing, for example, than in commercial) and the relative importance of the loss of a specific nutrient from a particular commodity (loss of vitamin C from milk during pasteurization and refrigerated storage, for example, is relatively unimportant, considering that milk is

a minor source of this nutrient in the daily diet, compared with other foods such as citrus fruits).

Adding to the evaluation process is the fact that food processing methods affect flavor, texture, and appearance. The processes that lead to improvements in these esthetic qualities (and consequently to reduced rejection of the food by finicky eaters) frequently lead to better retention of nutrients as well.

Processing Effects: Pluses and Minuses

Early man preserved his food supply by smoking, salting, and drying, and these basic methods are still utilized today. An analysis of these and other methods of food processing reveals both favorable and adverse effects on nutritional quality.

For example, on the positive side, heat processing destroys the antidiigestive factors in cereal grains, peas, and beans, thus making both the proteins and carbohydrates in these products more utilizable by man. Heat processing also destroys the enzymes which bring about the destruction of vitamin B₁ in fish and fish products, and the factors that would otherwise tie up the vitamins and iron in egg white.

In general, however, the overall effect of heat processing foodstuffs or drying them is to decrease the nutrient content, particularly vitamins A, B₁, C, and E. Heat processing or periods of long storage may also reduce protein availability, while drying—either alone or accompanied by smoking and storage—may reduce the stability of any fat components, leading to rancidity.

Processing Losses vs Natural Differences

Variations in the nutrient content of raw food materials will affect the content of vitamins and minerals in the final food product as much as—and sometimes *more* than—the processing itself. Raw foods may vary widely in their vitamin content because of genetic variations, climatic conditions, and maturity at harvest. This is especially true of the vitamin and mineral content of some fresh fruits and vegetables. These variations may be quite extreme—for example, carrots may vary 100-fold in their concentration of carotene (provitamin A), and samples of fresh tomato juice have shown 16-fold differences in vitamin C per serving. Similarly, investigators have found a wide range of thiamine concentration in pork muscle, depending in large part on the thiamine content of the diet the pigs received.

These natural differences are of long standing. Although the data are somewhat sketchy, it does not appear that the raw foods being produced today are any different (in terms of vitamin content) from those produced two or more decades ago.

Losses Occur During Cooking

Another factor which must be considered in evaluating nutrient losses resulting from food processing is the extent and kind of losses that occur during the preparation of the food for the table. Normal home cooking frequently leads to high losses of nutrients in food. In fact, the major loss in vitamin and mineral contents of foods often occurs during final preparation in the home or institution prior to eating.

Water-soluble vitamins, such as vitamin B₁, riboflavin, and niacin, are particularly subject to cooking losses through leaching. Losses in the fat-soluble vitamins and vitamin C, on the other hand, generally occur during heating and storage in the presence of air.

Because of the large losses that occur in the home, the actual vitamin content of table-ready foods is frequently about the same *regardless* of the type of processing—or lack of processing the food has under-

gone. For example, a bowl of peas placed steaming on the table will contain 35–45% of its original, “raw” vitamin C content regardless of whether it was prepared from fresh peas (45%), frozen peas (40%), or canned or freeze-dried peas (both 35%).

What Happens to Vitamins and Minerals?

The major methods of food preservation are blanching, heat processing, drying, freezing, and fermentation.

Blanching is the initial process in most preservation methods for fruits and vegetables. These foods are blanched to inactivate biological systems which would otherwise degrade the flavor or color, and the systems which cause the loss of vitamins.

Steam blanching of spinach, as an example, results in the retention of 90–100% of vitamins B₁, B₂, and C, and niacin. Water blanching (for 2½ to 5 minutes in boiling water) results in a retention of 65–90% of the same vitamins.

Minerals in food materials, on the other hand, are stable to heat. Any losses usually result directly from leaching into the water used for processing, or (in even larger amounts) into the water used for cooking.

Heat Processing includes canning in either metal cans or glass jars. Most vitamins, with the exception of riboflavin and niacin, break down when heated (i.e., are heat-labile). As a consequence, some nutrient losses can be expected in heating operations. Some vitamins, such as riboflavin, are also unstable with respect to light, significant losses of these materials may be expected in usual handling operations when the food material is exposed to both light and high temperatures.

Heat transfer is slow in conventionally sterilized products, particularly non-liquid products such as meat. Since heat is applied to the outside of the product (or its container), the outer material is subject to more total heating than necessary, in order to achieve sterility in the center. For example, in the canning of beans (a semi-solid), approximately 55% of the vitamin B₁ is retained, while in tomatoes (a more liquid product), approximately 70% of the vitamin C is retained.

High-temperature, short-time sterilization is receiving increased attention today because of the different effects of increased heat on bacterial destruction compared to chemical reactions. For example, an 18° F rise in processing temperature will usually produce a 10-fold increase in bacterial destruction, while only doubling the chemical reactions which lead to the destruction of certain vitamins and flavors. This system thus results in the retention of a higher percentage of nutrients and flavors than does conventional canning. Beans processed in this manner, for example, retain 90% of their original vitamin B₁, and tomato juice will retain an equivalent percentage of vitamin C.

Drying. The process of drying does not cause major losses in vitamins. This is true of conventional dehydration methods, and even more true for the newer methods such as puff drying and freeze drying.

Sulfur dioxide is frequently added in the dehydration process, with the primary intention of preserving the product's color. It also results in an *increased* retention of vitamin C, since sulfur dioxide inhibits a biological system which can cause a major loss of this vitamin. The addition of sulfur dioxide *does* cause a considerable loss of thiamine, but since most food products which are dehydrated and sulfured are not major sources of dietary thiamine, the net dietary effect of sulfur dioxide addition is positive.

When dried food products are stored in air, losses of vitamins A, C, and E may occur from reaction with oxygen.

Refrigeration and Freezing. The freezing process, like dehydration, does not in itself result in a significant destruction of vitamins, with the exception of vitamin E. Any losses in frozen foods occur during the blanching process prior to freezing, as noted above.

Fermentation. There is no major concern with nutrient losses with this method of preservation. In fact, there may be an *increase* in the B vitamins due to microbial synthesis during fermentation.

Storage. Vitamin losses during storage and distribution of canned or dried foods

may vary widely, depending on the temperatures at which they are held during the various stages of distribution. The retention of vitamins in canned tomato juice, for example, is very markedly decreased by storage at temperatures higher than room temperature, although vitamin A retention is somewhat less affected by storage conditions.

Storage temperatures are also important for the retention of quality, including nutritive value, in frozen foods. Thus, storage at 0° F or lower results in excellent retention of the vitamin content of frozen foods. The major factors affecting losses after long storage at these low temperatures are the oxygen permeability and light transmission characteristics of the packaging. At storage temperatures above 15° F, however, easily oxidizable vitamins will be lost over a period of time. For example, half of the original vitamin C in asparagus, peas, and lima beans will be lost during storage at 15° F for 6 months.

What Happens to Proteins?

Proteins in foods may become less available physiologically during processing or storage—that is, their molecular structure may change so that the body is less able to utilize them. Recent publications suggest that the mechanism by which these changes occur is a complex series of chemical reactions, some of which involve free radicals; these reactions occur much more readily in the presence of carbohydrates such as simple sugars.

Amino acids, the "building blocks" of proteins, may be destroyed or rendered non-utilizable when proteins are heated to high temperatures, and they may also be lost (at a slower rate) when foods are stored at room temperature.

The browning of food by heating or long storage may lead to loss in palatability and protein availability, as well as to the production of undesirable changes in the physical properties of food. On the other hand, most baked and fried foods are intentionally browned to "improve" their appearance and flavor. Maple syrup owes its flavor and color to the browning re-

action, and the distinctive caramel flavors which may be generated in dairy products are the result of the browning reaction in milk or milk components. Thus, while it is clear that browning may affect the nutritional value of foods, it may also enhance their acceptability.

What Happens to Carbohydrates and Fats?

As indicated earlier, carbohydrates may be made more digestible, and thus more available, by mild processing. The major loss in the availability of carbohydrates is due to their interaction with protein, as noted above. In most cases, even this loss is relatively minor, with more significant effects occurring in the protein constituents themselves.

Fats in foods are not significantly altered by processing, but may be degraded during prolonged storage in the presence of air.

Processing Does Not Cause Major Losses

On an overall basis, the food preservation techniques in greatest use today do not result in major losses in the nutritive value of foods, and the more sophisticated

methods of food preservation now being developed by advanced technology will retain an even higher percentage of nutrients. Factors to be considered in efforts to increase the retention of nutritional values must include the home preparation of food, institutional food systems, and further improvement in food processing technology. □

-
1. A. E. Bender: Nutritional effects of food processing. *J. Food Technol.* 1:261, 1966
 2. A. E. Bender: Nutritional effects of food processing. 1. Vitamin Losses. 2. Mineral salts, protein specific commodities. 3. Equipment and methods. *Rev. Nutr. Food Sci.* 11:2; 12:10; 13:6, 1968
 3. C. O. Chichester: Nutrition in food processing. *World Rev. Nutr. Diet.* 16:104, 1973
 4. D. F. Hollingsworth: Effects of some new production and processing methods on nutritive values. *J. Am. Diet. Assn.* 57:246, 1970
 5. D. F. Hollingsworth and P. E. Martin: Some aspects of the effects of different methods of production and of processing on the nutritive value of food. *World Rev. Nutr. Diet.* 15:1, 1972

Single copies of the Scientific Status Summaries and Reports may be obtained from the INSTITUTE OF FOOD TECHNOLOGISTS, 221 North LaSalle Street, Chicago, Illinois 60601, for 50 cents.

Fellowships in Clinical Nutrition

The Department of Nutrition and Food Science, Massachusetts Institute of Technology and the Children's Hospital Medical Center, Boston, Massachusetts, will offer fellowships in clinical nutrition starting July, 1975. The program is multidisciplinary in nature, providing physicians with an opportunity to acquire a broad background in clinical nutrition. The program's principal objective is the training of independent investigators for research in clinical nutrition and teaching of applied nutrition to house staff and medical students. The training program includes courses and seminars in nutrition at M.I.T. as well as clinical activities at Children's Hospital, the New England Deaconess and Boston Uni-

versity Hospitals. The fellowship will be offered for one, two or three years depending on the interests of the individual. One or two years training in pediatrics, internal medicine or surgery is required. Applicants must be citizens or non-citizen nationals of the United States, or have been lawfully admitted to the United States for permanent residence and have in their possession a permanent visa at time of application. Send all requests for information to Dr. Robert M. Suskind, Program Director, Clinical Nutrition Fellowship, Department of Nutrition and Food Science, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139. □

"Conquest of Famine" Receives Nutrition Foundations' Book Award

The Nutrition Foundation, the British Nutrition Foundation, and the Swedish Nutrition Foundation have selected *Conquest of Famine*¹ to receive the first Nutrition Foundations' Book Award.² The book by Dr. Wallace Aykroyd was published in England by Chatto & Windus Ltd. and in the United States by Readers Digest Press. Dr. Aykroyd was the Director of the Nutrition Division of the Food and Agriculture Organization from 1946 to 1960. He was Senior Lecturer in Nutrition at the London School of Hygiene and Tropical Medicine until his retirement in 1966.

It was during a symposium on famine sponsored by the Swedish Nutrition Foundation in 1970 that Aykroyd realized that a book dealing with the conquest of famine should be written in order to convey to the general reader the nature of this scourge, the causes of famine, the progress in the

procurement of food supplies and the organization of relief measures. *Conquest of Famine* describes famines from the potato famine in Ireland in the 1840's to the recent famine in Ethiopia, and contrasts these to the Biblical account of Joseph's preparation for the seven lean years of harvest in Egypt.

Dr. Aykroyd not only analyzes the social, environmental and political factors which contribute to famine and the various protocols for its alleviation, but he also leaves the reader with a feeling for the human misery which accompanies such deprivation. The author's assessment of the Green Revolution and new techniques of population control provides a mildly optimistic conclusion. He says, "what has been done in conquering famine in its traditional manifestations gives hope that man will successfully master the broader prob-

lem of adjusting world food supplies and world population."

As Aykroyd notes, "it has become fashionable to decry the Green Revolution, which has in fact been remarkably successful, though its achievements in India have been overshadowed by economic and social disruption accompanying the creation of Bangladesh, and by a grim drought in Western India." It is fortunate that there recently has appeared another most readable book, *Facing Starvation: Norman Borlaug and the Fight Against Hunger*, by Lennard Bickel.³ This book tells the personal story of the remarkable team from The Rockefeller Foundation, initially headed by J. George Harrar, and its campaign against hunger, first in Mexico and then around the world. It carries to a wide readership the story of the most recent developments, especially in relation to wheat, told around a central theme of the dedication of Nobelist Norman Borlaug.

The history of the agricultural program of The Rockefeller Foundation from its early days in Mexico in 1943 to 1966 was recorded earlier in *Campaigns Against Hunger*.⁴ This book was written by three other pioneers, E. C. Stakman, Richard Bradfield and Paul C. Mangelsdorf, who helped conceive and develop this program.

These three books well could be considered required reading during the World Food and Population Year of 1974-1975.

1. W. Aykroyd: *The Conquest of Famine*. Chatto & Windus Ltd., London, 1974
2. International Group of National Nutrition Foundations. *Nutrition Reviews* 31:35, 1973
3. L. Bickel: *Facing Starvation: Norman Borlaug and the Fight Against Hunger*. Readers Digest Press, 1974. Available from E. P. Dutton & Co., Inc., New York
4. E. C. Stakman, R. Bradfield, and P. C. Mangelsdorf: *Campaign Against Hunger*. Harvard University Press, Cambridge, Massachusetts, 1967

Conquest of Famine is available from E. P. Dutton & Co., Inc., 201 Park Avenue South, New York, New York 10003, for \$7.95.

Effects and Metabolism of Glucose Tolerance Factor

by Walter Mertz, M.D.

The immediate history of the glucose tolerance factor (GTF) began with the observation that feeding rats a *Torula* yeast based diet resulted in a significant impairment of their glucose tolerance, as measured by intravenous glucose tolerance tests.¹ Feeding this diet also produced dietary necrotic liver degeneration, but none of the nutrients protecting against the liver disease (vitamin E, sulfur amino acids, factor 3 preparations, or selenium) were effective in preventing the decline of glucose tolerance.² The existence of a new dietary agent, GTF, was postulated,³ trivalent chromium was identified as its active ingredient,⁴ and its mode of action was described as facilitating the reaction of insulin with receptor sites of sensitive tissues.⁵ The best known and richest source of GTF in nature is Brewer's yeast.

In retrospect, the history of GTF may date back to 1929, when E. Glaser and G. Halpern described an insulin potentiating effect of yeast extracts by demonstrating that incubation of insulin with such extracts would result in a substantially greater hypoglycemic action of the hormone.⁶ This exciting work was not followed up,

probably because the results were conveniently explained by the high vitamin content of yeast, and they were overshadowed by the great therapeutic benefits of insulin itself. With this died an art of treating diabetes by nutritional means, going far beyond the regulation of energy intake, to the prescription of certain foods and waters that had been shown by experience to be beneficial to diabetics.

The mid-sixties brought the demonstration of improvement of glucose tolerance in a number of adults and elderly people in the United States, following supplementation with 150 to 250 μ g of chromium per day, in the form of chromic chloride.⁷⁻⁹ Shortly thereafter, very marked effects of such supplementation were found in malnourished children from Jordan, Nigeria, and Turkey, but not from Egypt.¹⁰⁻¹² At the same time, however, it was concluded from animal experiments that simple chromium salts, regardless of valence state, did not meet the criteria of an essential element.⁵ Most importantly, a variety of these salts were shown not to have access to the fetus in utero, although it was known from careful analytical studies that the fetus acquires high concentrations of chromium during its development. Second, intestinal absorption of simple chromium compounds was not found to depend on the nutritional chromium status of the animal, and the rate of chromium elimination not to be influenced by superimposed injections of high doses of chromium chloride.¹³ Third, the effects of chromic chlo-

Dr. Mertz is Chairman of the Nutrition Institute, Agricultural Research Service, Beltsville, Maryland 20705.

A limited number of reprints of this article may be obtained from the author. THERE ARE NO REPRINTS OF UNSIGNED REVIEWS.

ride supplementation in adult man always took several days to several weeks to be noticeable, and this interval could not be shortened by giving higher doses. Furthermore, simple chromium compounds were shown not to equilibrate with a physiologically important pool in the organism, the source of pronounced, acute increases of blood chromium in response to increases of circulating insulin.^{14*} Finally, calculations of the approximate chromium balance in man indicated a very strong deficit when the absorption rate for chromic chloride is assumed to be the same as for food chromium.¹⁴ For example, this rate of absorption of the average dietary chromium intake of 50 to 100 μg per day in the U.S. would supply only 0.25 to 0.5 μg of the 7 to 10 μg that are excreted daily in the urine. Such an imbalance would result in depletion of the chromium stores within a few years, incompatible with the presence of measurable chromium concentrations in the tissues throughout life.

This apparent contradiction can be resolved by postulating that certain chromium compounds in foods must be absorbed better, be transported in a different way, and have access to body pools inaccessible to "simple" chromium compounds. Experiments with extracts from Brewer's yeast grown in a ^{51}Cr -enriched medium suggested that the yeast was able to transform the "inorganic" chromium chloride into a form that more nearly met the criteria of an essential element. As an example, one of the earliest experiments established that inorganic chromium, added to chromium-depleted Brewer's yeast significantly stimulated the rate of CO_2 production, but only after a lag phase of 3 to 16 hours. When extracts of Brewer's yeast that had been allowed to accumulate chromium, were added to chromium-depleted yeast, the

stimulation of CO_2 production appeared immediately.²⁰ Subsequently, ^{51}Cr extracted from Brewer's yeast was shown to be better absorbed than chromic chloride by the rat, to be transported across the placenta, to have a different tissue distribution, and to have access to that pool that is the source of the acute plasma increment in response to insulin.

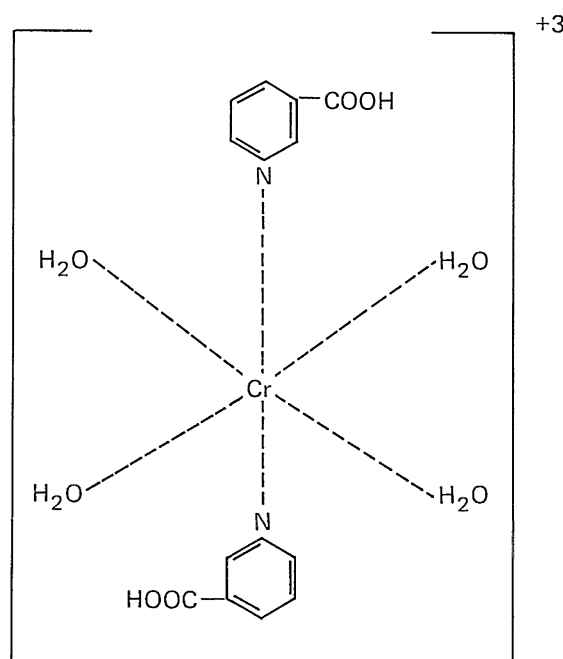
These observations were the basis for the theory that there exist two categories of chromium compounds in nature. The first consists of "simple" compounds, for example, chloro, aquo, or acetato coordinate compounds or complexes, as well as a large number of complexes having organic acids, amino acids, sugars, or certain vitamins as ligands. These can have a measured degree of insulin potentiating activity *in vitro*, but they do not meet the criteria of an essential element discussed above. The second category consists of compounds with outstanding insulin potentiating activity *in vitro* and with a metabolic behavior described above for yeast chromium. This latter category is termed glucose tolerance factor; it may consist of one or several closely related compounds. Attempts in the past to find known or newly synthesized compounds with GTF activity were consistently unsuccessful. Therefore, it was attempted to concentrate, purify, isolate, and identify the chromium compound(s) from the richest known source of GTF: Brewer's yeast. A relatively simple procedure was developed which not only concentrated chromium, but also biological activity, as measured by the potentiation of insulin in chromium-deficient rat epididymal adipose tissue *in vitro*.²¹

The results of this work consistently pointed to a substance of low molecular weight, water soluble, heat stable in solution, and absorbing at 262 nm, as being associated with biological activity and with part of the chromium content of the preparations. Nicotinic acid was identified by mass spectrometry as the compound associated with chromium. Chromium-nicotinic acid complexes were synthesized. They exhibited the same, strong insulin potentia-

*This increase, a very important part of chromium metabolism, has been observed by several independent investigators.¹⁴⁻¹⁷ Others, using a direct method of chromium determination in the graphite furnace, have reported the opposite: a decrease of chromium concentrations, following a glucose challenge.^{18,19} For reasons to be discussed later, this reviewer accepts the increase as real.

ting effects as the extracts from Brewer's yeast. A method for synthesizing these compounds from chromic acetate and niacin in 80 percent ethanol has been described. The reaction product is purified by adsorption on a cation exchanger and subsequent elution by NH_4OH . The ammonia eluate contains a substance or a mixture of substances of intense blue-violet color with a strong absorption of 262 nm.²² The color develops only after boiling the reaction mixture, and it is distinctly different from that of chromic acetate. This indicates complex formation, and the presence of the 262 nm absorption band in the colored compound after several separation steps is indicative of a chromium-nicotinic acid complex. The exact structure has not been identified yet but, in analogy to well known nicotinic acid complexes of other transition metals, is probably that of a tetra-aquo, di-nicotinato chromium (III) complex of the hypothetical structure shown in Figure 1.

Figure 1. Tetra-Aquo-Di-Nicotinato Chromium Complex



As is true for most aquo complexes of chromium, this compound is unstable in

solution, except at acidic pH. In a near neutral milieu it begins to precipitate and, with time, turns into an insoluble substance of greenish-grey color, distinctly different from that of the original complex. This process is caused by hydrolysis of the coordinated water molecules and subsequent formation of OH-Cr-OH bridges (olation), leading to the formation of large macromolecules.⁵ Before becoming insoluble, the complex (or mixture of complexes) has distinct biological activity in the epididymal fat pad system. Even with this relatively simple structure, and assuming that only the complex represented in Figure 1 is formed, there are two possible isomers, a cis and a trans form. In view of the stability of many stereoisomers of chromium complexes, it is likely that these two isomers will have different biological properties.

Although the niacin-chromium-niacin coordination imparts high biological activity to the metal, there is no known mechanism by which such a complex could exist at the alkaline pH of the organism for more than a few hours. Stabilization can be achieved by replacing the coordinated water molecules with ligands not undergoing olation. Analysis of highly purified GTF preparations from yeast had indicated the presence of glycine, glutamic acid, and a sulfur containing amino acid. Reacting these amino acids with the preformed chromium-niacin complex resulted in compounds of great stability against alkali and heat, and of biological activity equal to or greater than that of the tetra-aquo, di-nicotinato chromium complex. Other amino acids replacing the water molecules have given similar stability without, however, yielding complexes of the same high biological activity. Although the reaction product is further purified by ion exchange chromatography, as described above, it is most likely a mixture of closely related compounds, as there are numerous ways in which five ligands can be coordinated. Attempts to isolate and crystallize one well defined component have not yet been successful.

Biochemistry of Natural and Synthetic Compounds with GTF Activity

The behavior of the synthetic compounds during purification is similar or identical to that of purified fractions from Brewer's yeast containing GTF activity. For example, both preparations are retained by Dowex-50 resin, and most of the biological activity and part of the total chromium is eluted by NH_4OH . A second fraction containing most of the chromium but little biological activity, is eluted by concentrated acid. The fractions containing high biological activity, natural or synthetic, have the same elution volume upon gel filtration and the same R_f upon paper chromatography. Finally, the elution patterns of ^{52}Cr in yeast extracts and of ^{51}Cr in synthetic complexes from cation exchange resins are nearly identical.²² That the strong potentiation of insulin *in vitro* depends on the coordination of nicotinic acid to chromium is shown by the ineffectiveness of other pyridine carboxylic acid derivatives as ligands, and of other transition elements as substitutes for chromium. Nicotinic acid by itself has a measured degree of activity *in vitro*, but significantly less than the chromium complex.²³

A very important development, extending beyond the previously known effects of GTF in chromium-deficient animals is the recent observation that GTF concentrates from Brewer's yeast significantly reduce the elevated blood levels of glucose, triglycerides, and cholesterol of genetically diabetic mice, upon intraperitoneal injection.²⁴ Synthetic chromium-niacin complexes were also shown to lower blood glucose levels in these animals; the onset of the effect was observed after only one hour, as compared to four hours for the yeast concentrates.²⁵ Experiments to determine the effect of the synthetic complexes on serum triglycerides and cholesterol are not yet complete.

The best known mode of action of compounds with GTF activity, synthetic or derived from yeast, namely the potentiation of insulin, is postulated to be mediated through the formation of a ternary com-

plex between chromium, insulin, and insulin receptors of cell membranes. This mechanism would explain all of the known effects of GTF in insulin responsive systems. While this ternary complex is hypothetical there does exist experimental proof for the formation of complexes between insulin and natural, as well as synthetic GTF compounds. These form and are quite stable at alkaline pH and can be separated from free GTF by gel filtration²⁶ or precipitation. These findings, of course, do not exclude other potential modes of action of inorganic or GTF chromium.

Metabolism of Glucose Tolerance Factor

As discussed previously, GTF chromium, as produced by Brewer's yeast, differs in its metabolism from simple chromium compounds in intestinal absorption, tissue distribution, access to a special chromium compartment, and placental transport. Synthetic chromium-niacin complexes, although having equal or better insulin potentiating activity *in vitro* than yeast extracts, are not fully equivalent to the latter in their metabolic behavior. Although they are better absorbed by a factor of three or four than are simple chromium compounds, they fall short of the 10 to 25 percent absorption of yeast extracts. The synthetic complexes have a tissue distribution different from that of simple chromium compounds, but not identical to that of yeast chromium. They do have access to the special compartment which is the source of the acute plasma chromium increment in response to insulin, similar to yeast chromium.²⁷ The question of placental transport is not yet resolved. These observations are consistent with the hypothesis that the synthetic preparations now available are mixtures of closely related compounds of which only one behaves like yeast chromium, whereas the others do not and may even mask the effect of the former.

The synthetic complexes are utilized as well as free nicotinic acid by the niacin-deficient rat, with approximately 15 mg of

the vitamin per kilogram of diet producing near maximal growth effects, whether free or bound in the complex. Niacin by itself and within a physiological range of concentrations does not affect glucose tolerance of rats, whereas the complex does. The toxicity of the complex is low. The acute LD_{50} , upon intravenous injection in rats is 1 g per kilogram of body weight, corresponding to approximately 60 mg of chromium. This compares to an LD_{50} of 18 mg chromium as chromalum. Experiments attempting to demonstrate chronic toxicity of the synthetic compounds (up to 4.6 g per kilogram of diet) are in progress, but have not yet produced adverse signs.²⁸

Analysis of Chromium

In view of the great differences in absorbability, metabolism, and biological action between the two categories of chromium compounds, even a reliable analysis of total chromium in tissues has little value to the nutritionist. Unfortunately, reliable and universally accepted methods to determine chromium in biological materials do not yet exist, in spite of considerable effort by organizations such as the International Atomic Energy Agency, the World Health Organization, or the National Bureau of Standards. It appears that each substrate presents its own analytical problem, predominantly related to sample preparation. The biological substrates that present the greatest difficulties are those with substantial GTF content such as yeast, liver, and meats, whereas materials with little GTF activity, such as the National Bureau of Standards' orchard leaves, are analyzed much more easily and with more agreement among different methods. It is not known what the reasons for these difficulties are. Volatility of chromium in certain foods during oven drying at 80°C has been reported,²⁹ but the formation of refractory species cannot be ruled out. One interlaboratory comparison reported chromium concentrations in one standard tissue that differed by a factor of thirty.³⁰ Ten- and three-fold differences of results have been obtained

under controlled conditions in one laboratory for molasses³¹ and urine,³² respectively, depending on the method of digestion.

There is a method, however, to estimate the state of chromium nutrition of individuals with some confidence. As the chromium absorbed into the organism is almost entirely excreted in the urine,³³ the urinary chromium loss furnishes a rough estimate of an abnormal metabolism. In the experience of this reviewer, chromium in the urine can be determined accurately if the analysis is preceded by careful ashing in an activated oxygen medium. The daily excretion is abnormally high in diabetic children treated with insulin,¹⁶ and abnormally low in Turkish subjects living in an area of suspected chromium deficiency.³⁴ More important, perhaps, than the daily loss is the presence or lack of an acute increase in urinary chromium excretion following an oral glucose load. This increase which probably reflects the increase of chromium in blood, can be detected by careful analysis in chromium-sufficient subjects,^{32,36} but not by direct application of the sample to the graphite furnace. The lack of this increment suggests exhaustion of the stores of biologically important chromium in the organism; its presence indicates an acceptable nutritional status. Similar to the controversy concerning the acute chromium increment in blood, there are conflicting views as to urinary chromium, following a glucose challenge.^{32,37} These apparent discrepancies emphasize the need for a standardized method of chromium analysis.

Conclusion

This review reveals the many gaps in our knowledge of chromium metabolism. These can best be summarized in an attempt to classify the biologically active form of this element, GTF, according to accepted categories. While GTF is an essential micro-nutrient, it does not fit the description of either a typical trace element or a typical vitamin. It differs from other trace elements in the strict dependence of its action

and availability on the chemical structure in which the element is bound. GTF may be a vitamin for the pregnant rat which depends on it for placental transport, and perhaps for some adult human subjects who cannot utilize inorganic chromium, but not for the malnourished child who immediately responds to chromic chloride. Chromium in its biologically active form resembles a hormone, by being released into the blood in response to a physiological stimulus (insulin) and being transported to the periphery where it exerts a marked biological action by facilitating a reaction which, in its absence, would occur at a much lower rate. A very powerful regulator of carbohydrate metabolism can be extracted from the liver, but not from other organs of rats, after a glucose challenge,³⁵ and these extracts have biological and chemical properties very similar to natural and synthetic GTF preparations. From these considerations the following hypothesis can be developed as a summary of this review. Glucose tolerance factor occurs preformed in certain foods and can be utilized directly by animals and man. Man has a varying ability to synthesize GTF from inorganic chromium, niacin, and amino acids, with the result that different subjects depend on preformed GTF to a different degree. The site of synthesis may be the intestinal flora or a special tissue in the organism, possibly the liver. In response to acute increases of insulin in the blood, GTF is released and exerts its action, the potentiation of insulin at the target organs. □

1. W. Mertz and K. Schwarz, *Arch. Biochem. Biophys.* 58: 504-506, 1955
2. W. Mertz and K. Schwarz, *Am. J. Physiol.* 196: 614-618, 1959
3. K. Schwarz and W. Mertz, *Arch. Biochem. Biophys.* 72: 515-518, 1957
4. K. Schwarz and W. Mertz, *Arch. Biochem. Biophys.* 85: 292-295, 1959
5. W. Mertz, *Physiol. Rev.* 49: 163-239, 1969
6. E. Glaser and G. Halpern, *Biochem. Z.* 207: 377-383, 1929

7. W. H. Glinsmann and W. Mertz, *Metabolism* 15: 510-515, 1966
8. R. A. Levine, D. H. P. Streeten, and R. J. Doisy, *Metabolism* 17: 114-125, 1968
9. L. L. Hopkins, Jr. and M. G. Price: Effectiveness of Chromium (III) in Improving the Impaired Glucose Tolerance of Middle-Aged Americans. Western Hemisphere Nutrition Congress, vol. II, pp. 40, Puerto Rico, 1968
10. L. L. Hopkins, Jr., O. Ransome-Kuti, and A. S. Majaj, *Am. J. Clin. Nutrition* 21: 203-211, 1968
11. C. T. Gürson and G. Saner, *Am. J. Clin. Nutrition* 24: 1313-1319, 1971
12. J. P. Carter, A. Kattab, K. Abd-El-Hadi, J. T. Davis, A. El Cholmi, and V. N. Patwardhan, *Am. J. Clin. Nutrition* 21: 195-202, 1968
13. W. Mertz, E. E. Roginski, and R. C. Reba, *Am. J. Physiol.* 209: 489-494, 1965
14. W. Mertz and E. E. Roginski in *Newer Trace Elements in Nutrition*. W. Mertz and W. E. Cornatzer, Editors, pp. 123. Marcel Dekker, New York, 1971
15. W. H. Glinsmann, F. J. Feldman, and W. Mertz, *Science* 152: 1243-1245, 1966
16. K. M. Hambidge in *Newer Trace Elements in Nutrition*. W. Mertz and W. E. Cornatzer, Editors, pp. 169. Marcel Dekker, New York, 1971
17. D. Behne and F. Diehl in *Nuclear Activation Techniques in the Life Sciences*. Pp. 407. International Atomic Energy Agency, Vienna, Austria, 1972
18. I. W. F. Davidson and R. L. Burt, *Am. J. Obstet. Gynecol.* 116: 601-608, 1973
19. R. S. Pekarek, E. C. Hauer, E. J. Rayfield, R. W. Wannemacher, Jr., and W. R. Beisel, *Fed. Proc.* 33: 660, 1974
20. J. N. Burkeholder and W. Mertz in *Proceedings of the Seventh International Congress of Nutrition*. Vol. V, pp. 701. Pergamon Press, New York, 1967
21. W. Mertz, E. W. Toepfer, E. E. Roginski, and M. M. Polansky, *Fed. Proc.* 33: 2275-2280, 1974
22. M. M. Polansky, *Fed. Proc.* 33: 659, 1974
23. E. E. Roginski, *Fed. Proc.* 33: 659, 1974
24. R. W. Tuman and R. J. Doisy in *Trace Element Metabolism in Animals-2*. W. G. Hoekstra, J. W. Suttie, H. E. Ganther, and W. Mertz, Editors, pp. 678. University Park Press, Baltimore, 1974
25. R. W. Tuman and R. J. Doisy, abstract, 35th Annual Meeting of the American Diabetic Association, 1975 (submitted)

26. G. W. Evans, E. E. Roginski, and W. Mertz, *Biochem. Biophys. Res. Commun.* 50: 718-722, 1973
27. W. Mertz, *Fed. Proc.* 33: 659, 1974
28. W. Mertz and E. E. Roginski, *Fed. Proc.* 34, 1975 (abstracts)
29. V. Maxia, S. Meloni, M. A. Rollier, A. Brandone, V. N. Patwardhan, C. I. Waslien, and S. W. Shami in *Nuclear Activation Techniques in the Life Sciences*. Pp. 527. International Atomic Energy Agency, Vienna, Austria, 1972
30. WHO/IAEA Joint Research Programs of Trace Elements in Cardiovascular Disease. Research Coordination Meeting, Vienna, Austria, 1973
31. W. R. Wolf, W. Mertz, and R. Masironi, *J. Agr. Food Chem.* 22: 1037-1042 1974
32. W. R. Wolf, F. E. Greene, and F. W. Mitman, *Anal. Biochem.* (submitted)
33. R. J. Doisy, D. H. P. Streeten, L. M. Souma, M. E. Kalafer, S. I. Rekan, and T. G. Dalakos in *Newer Trace Elements in Nutrition*. W. Mertz and W. E. Cornatzer, Editors, pp. 155. Marcel Dekker, New York, 1971
34. C. T. Gürson, G. Saner, W. Mertz, W. R. Wolf, and S. Sökücü, *Nutrition Rep. Int.* (submitted)
35. W. Mertz and K. Schwarz, *Am. J. Physiol.* 203: 53-56, 1962
36. H. A. Schroeder, *Am. J. Clin. Nutrition* 21: 230-244, 1968
37. I. W. F. Davidson, R. L. Burt, and J. C. Parker, *Proc. Soc. Exp. Biol. Med.* 147: 720-725, 1974

Nutrition Reviews

authoritative articles by distinguished international scientists • competent, critical, and knowledgeable reviews • interpretative summaries of important original clinical and experimental research • selected excerpts from classic publications marking milestones in nutrition research • informative special reports on government and organization actions • contributing editors review journals of widespread interest covering biochemistry, food science, public health, toxicology, dentistry, pediatrics, internal medicine, gastroenterology and other clinical specialties •

Send subscription and renewal requests to:

THE NUTRITION FOUNDATION
489 Fifth Avenue
New York, New York 10017

\$12.00 per year

\$6.00 for students

DIET, INTESTINAL FLORA, AND COLON CANCER

The daily fecal excretion of neutral sterols and of bile acids is much higher in people consuming a typical American diet than in vegetarians. Most of the sterols in typical Americans are converted to other products by bacterial action.

The degree of conversion, however, is limited in some individuals and apparently not related to diet.

Key Words: diet, sterols, bile acids, carcinogens, colon cancer

The incidence of cancer of the colon varies greatly in different populations. Generally speaking the incidence is high in economically favored populations who consume diets high in animal products, fat, sugar, and processed foods and low in those populations consuming high cereal diets. D. P. Burkitt¹ developed the thesis that these differences may be explained on the basis of the fiber content of the diets. A high fiber diet produces a larger volume of stools and stimulates the passage of material through the intestine. Carcinogenic materials in the intestine would thus have a shorter contact time and less opportunity to promote the development of cancer.

An alternative or supplementary thesis is that the high meat-low fiber diet results in the production of more carcinogens in the lumen contents, possibly derivatives of sterols and bile acids. The data of M. J. Hill et al.² suggested a correlation between high concentrations of neutral sterols and bile acids in the feces and the incidence of colon cancer. Although some bile acids have produced sarcomas at the site of injection,^{3,4} there is also evidence that intestinal microflora might produce carcinogens from bile acids. Thus, the nature of the diet influences both the type and number of the intestinal flora. The derivatives of bile acids and sterols produced by the microflora may also be etiologic factors in cancer of the colon.

B. S. Reddy and E. L. Wynder⁵ investigated the amount and composition of the fecal sterols and bile acids in several United States populations with rather different dietary habits. These included Americans consuming the usual diet, so-called vegetarians consuming diets without meat but which did contain milk, Seventh-Day adventists also consuming a largely vegetarian diet but with milk and eggs, recent Japanese migrants in the United States still consuming a more or less typical Japanese diet which contains fish but only small amounts of meat, milk, and eggs, and recent Chinese migrants also consuming a diet low in animal products. The sterols measured were cholesterol, coprostanol, and coprostanone—the latter being microbial degradation products of cholesterol. The total of these fecal sterols was much higher in typical Americans, 817 mg per day on the average. The vegetarians produced a total of 318 mg per day and the other groups still less with the Chinese producing the least, 195 mg per day. In contrast the total cholesterol in the feces of the typical Americans was the least, only 30 mg per day. Thus, although the total sterol in the feces of the typical Americans was much higher than in the other groups, most of it had been converted to coprostanol and coprostanone. The authors compute the percent of cholesterol degraded as

$$\frac{\text{coprostanol} + \text{coprostanone}}{\text{total neutral sterol}}$$

In the typical Americans this yields a value of 96 percent and ranges downward in the other groups to 46 percent in the Japanese.

Cholic acid, deoxycholic acid, and lithocholic acid were estimated. The amounts of all three bile acids were higher in the feces of the typical Americans with a total of 256 mg per day.

The authors also measured the amount of microbial beta-glucuronidase in the feces. This enzyme is not thought to have any particular function related to carcinogenesis but was used simply as an indicator of the amount of microbial activity. The activity in the feces of those consuming the typical American diet was much higher than in the other groups and ranged down to the lowest values in the feces of the Japanese and the Chinese.

Thus, it is concluded that the data are at least consistent with the hypothesis that large amounts of intestinal sterol and bile acids are produced by the American diet and there is extensive microbial action which modifies sterols and might produce carcinogenic materials.

An interesting extension of these kinds of studies has been reported by T. D. Wilkins and A. S. Hackman.⁶ They studied the fecal neutral sterols in 31 volunteers using gas liquid chromatography which allowed the estimation of both cholesterol and the plant sterols, sitosterol and campesterol, and their degradation products. The 31 subjects could be divided into two rather well-defined groups. One group containing 23 subjects was characterized by extensive conversion of cholesterol, sitosterol, and campesterol by the intestinal flora while the other group was characterized by little or no conversion. For example, of the high converters, all converted more than 60 percent of the cholesterol and 13 of the 23 converted more than 90 percent. Of the low converters, seven converted less than 20 percent.

Sixteen of the subjects—ten high converters and six low converters—were followed over periods up to 22 months to examine the stability of the sterol pattern in the feces. One of the low converters, on the basis of the initial examination, became a

high converter and occasional samples in other subjects were not consistent with the usual findings. Yet it seems fairly clear from the data that over this period most of the subjects remained either high or low converters of the intestinal sterols.

No dietary data are reported for these subjects. It is stated that "All were on a normal American diet." The fact that the total sterols of plant and animal origin were approximately the same in the feces of the two groups indicates that their diets were of similar composition.

Thus, although it is clear that the nature of the diet affects the microflora of the intestine and these may have beneficial or detrimental effects, the differences within population groups point to a relationship that is more complex than might be suspected.

No doubt the etiology of cancer of the colon when it is understood will prove to be complex. The epidemiologic evidence implicating the diet is strong. The two primary hypotheses at the moment, one attributing a preventive role to diets high in dietary fiber, and the other attributing a casual role to high intakes of animal products, are both consistent with the evidence and the practical implications are the same. □

1. D. P. Burkitt: Epidemiology of Cancer of the Colon and Rectum. *Cancer* 28: 3-13, 1971
2. M. J. Hill, B. S. Draser, G. Hawksworth, and V. Arees: Bacteria and Etiology of Cancer of the Large Bowel. *Lancet* 1: 95-100, 1970
3. J. W. Cook, E. L. Kennaway, and N. M. Kennaway: Production of Tumors in Mice by Deoxycholic Acid. *Nature* (London) 145: 627, 1940
4. A. Huddow: Chemical Carcinogens and Their Modes of Action. *Brit. Med. Bull.* 14: 79-92, 1958
5. B. S. Reddy and E. L. Wynder: Large-Bowel Carcinogenesis: Fecal Constituents of Populations with Diverse Incidence Rates of Colon Cancer. *J. Nat. Cancer Inst.* 50: 1437-1442, 1973
6. T. D. Wilkins and A. S. Hackman: Two Patterns of Neutral Steroid Conversion in the Feces of Normal North Americans. *Cancer Res.* 34: 2250-2254, 1974

STUDIES ON SELENIUM

The principal sources of selenium in the human diet are cereals, followed by meat, poultry, and fish, and then the dairy products. Selenium is incorporated into the teeth either from inorganic or organic compounds with the major amount being found in the protein matrix of the enamel and dentin.

Key Words: selenium, human foods, tooth development, dental caries

Current interest in selenium varies from concern about the adequacy of human diets for all age groups to the desire to know whether slightly elevated levels of selenium ingestion may alter the formation of the teeth to cause them to be more susceptible to dental caries.

In an effort to evaluate the amount of selenium available for consumption, J. N. Thompson and co-workers determined the selenium content of food groups and composite diets based on the average distribution of foods consumed by Canadians.¹ Samples of 86 individual foods in Winnipeg and in Halifax and two samples of each in Toronto were purchased in proportion to their per capita rate of disappearance from the market place on a nationwide basis. The foods were trimmed, cooked in typical ways, and pooled in the appropriate proportions into the following 11 groups: dairy products; meat, poultry, and fish; cereal products; potatoes; leafy vegetables; legumes; root vegetables; garden fruits; other fruits; oil and fats; and sugar and adjuncts. The daily selenium contribution to the diet was determined for each of these food groups. In addition, a composite diet was prepared by mixing the 11 groups in the appropriate amounts for Winnipeg and Halifax and two composite diets in the same way for Toronto. The four composite diets were also analyzed for selenium. The authors also calculated the selenium content of the "average" Canadian diet from published values of selenium concentration in Canadian foods and the per capita rate of disappearance of fresh foods. Water and

other drinks were omitted from all of these comparisons on the basis that the authors considered these fluids to supply negligible amounts of selenium. This decision may be entirely appropriate, presuming that the water supplies in Winnipeg, Halifax, and Toronto are known to contain negligible amounts of selenium. However, in areas where communal water contains selenium in appreciable amounts, the amount from this source in the human dietary would also need to be calculated.

The calculations based on previously available data yielded food consumption of 1,646 g per person per day, providing 196.6 μg selenium per day. The four composite diets weighed 1,570 to 1,659 g per person per day. Analyses of the selenium contents of the 11 food groups used to make the composite diets from Winnipeg, Halifax, and Toronto #1 and #2 indicated 180.8, 224.2, 98.3, and 148.5 μg selenium per daily portion, respectively. The variation from diet to diet, especially between the two Toronto diets, was surprisingly high since they were composite diets. These selenium values vary from 0.06 to 0.14 μg per gram of fresh diet.

The largest amount of selenium in all four diets was provided by the cereal products in Group 3, next by the meat, poultry, and fish in Group 2, and then by the dairy products in Group 1. The dairy products in the Winnipeg and Halifax diets provided four to five times the amount of selenium as in the two Toronto diets. In addition, the meat, poultry, and fish in the Winnipeg and Halifax diets contained two to three times as much selenium as the Toronto diets. The other eight food group-

ings provided rather trivial amounts of selenium. The total values for the composite diets compared fairly well with the average daily selenium intake in the northeastern United States of 60 to 150 μg which was reported by H. A. Schroeder and co-workers.²

The possibility of a selenium deficiency in Canadian adults was considered by these authors to be remote. They expressed concern, however, that deprivation in infancy was possible due to the low amounts of selenium in milk and other dairy products.

Shearer studied the uptake of inorganic and organic selenium from drinking water by the fully developed molar teeth and the developing incisor teeth of pregnant rats and by the developing molar and incisor teeth of their offspring.³ Selenium was provided from the tenth day of pregnancy to parturition either as an 0.2 ppm solution of selenomethionine to ten rats or of sodium selenite to nine rats tagged in both instances with ^{75}Se . Distilled water was provided to the rats from parturition for 13 days at which time the dams and their offspring were sacrificed and samples prepared for selenium analyses. The time of sacrifice was determined so that the first and second molars of the offspring would not have erupted. For the rats provided with selenomethionine, 22.1 percent of the ingested dose was retained with one-half in the carcasses of the mothers and one-half in the offspring; for the rats fed sodium selenite, only 14.1 percent of the ingested dose was retained with slightly more than half in the bodies of the mothers.

In the rats provided with selenomethionine, the developing molars of the offspring incorporated almost eight times as much selenium on a weight basis as the fully-formed molars of the mothers. This ratio is not surprising due to the rapid development of molars in the offspring of this age and the lack of molar development in the mothers. The developing incisors of the offspring had incorporated almost identical amounts of selenium to the molars; the ratio of selenium in the offspring's incisors to that in their mothers' incisors was 1:6.

The incisors of the mothers, however, contained over five times the selenium of their molars. The latter observation is an interesting confirmation that the incisor of the adult rat is continuously erupting as enamel and dentin are being formed throughout life at the germinative center.

Among the rats provided with sodium selenite the incorporation of selenium into the molars of the offspring was only half that observed with selenomethionine; the amount incorporated into molars of the mothers was about half that in the offspring. The ratio of selenium in the incisors was about 2.0 times in the offspring for the amount in the mothers. Both values were substantially higher for incisors than for molars in the same rats, but were a little lower than for incisors in the rats provided with selenomethionine.

Among both the mothers and their offspring provided with either selenomethionine or sodium selenite, the highest percent of the ingested dose per 100 g of tissue was found in the liver and kidney, followed by spleen, heart, blood, and lungs. The authors stated that the selenium levels in milk in the stomachs of the offspring were routinely low, suggesting that the selenium had come from placental rather than mammary transfer. Except for teeth and bone, more selenium was present in all maternal tissues than in those of the offspring.

Both enamel and dentin from developing and developed teeth incorporated selenium either from selenomethionine or from sodium selenite. In all comparisons dentin values were higher than enamel. The incorporation of selenium into both enamel and dentin of developing molars was significantly higher from selenomethionine than from sodium selenite, while the reverse was true postdevelopmentally.

When selenium was provided as selenomethionine, over 79 percent of the total selenium in the developing enamel was located in the protein fraction, which is only about 2 percent by weight of the enamel; almost 95 percent of the selenium in dentin of these developing teeth was in the protein

fraction. In developed molars of the mothers, almost as high a percent of selenium was incorporated from selenomethionine into the protein fraction. When selenium was provided as sodium selenite, comparable amounts were incorporated into the protein fractions of enamel and dentin from developing teeth as was observed with selenomethionine. However, postdevelopmentally much less selenium was incorporated into the protein fraction of either enamel or dentin when sodium selenite was the source.

The selenium in the protein fraction was present either in the form of selenotrisulfides (R-S-Se-S-R) which were removed by treatment with dilute sodium hydroxide and mercaptoethanol, or in a more stable form which could not be removed from the enamel and dentin proteins under the conditions of the experiment. In developing enamel and dentin, 41 and 66 percent of the total selenium was in this stable form when selenomethionine was fed and 40 and 55 percent when sodium selenite was provided. Postdevelopmentally the values were similar for enamel and dentin after selenomethionine administration but were much lower when sodium selenite was given.

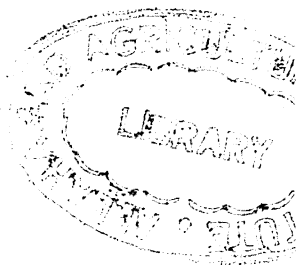
The results in this paper for sodium selenite in drinking water are very similar to those obtained by Shearer and Hadjimarkos when sodium selenite was injected subcutaneously four times during the last 12 days of pregnancy.⁴

The author did not study mineralization of the teeth in the offspring in this experiment or carry any of the offspring to an age when their caries susceptibility could have been determined. These areas need further careful investigation. He draws attention to the three epidemiological surveys in the western United States⁵⁻⁷ and

the additional one in Russia⁸ where an association between either the selenium concentration in drinking water or in urine or in teeth and dental caries was reported. In addition, an experiment with monkeys supplemented with selenium indicated an increased caries activity.⁹ He postulated that increased selenium, in the protein fraction of enamel especially, may alter the quality of mineralization and therefore the caries susceptibility of the teeth. □

1. J. N. Thompson, P. Erdody, and D. C. Smith: Selenium Content of Food Consumed by Canadians. *J. Nutrition* 105: 274-277, 1975
2. H. A. Schroeder, D. V. Frost, and J. J. Balassa: Essential Trace Elements in Man: Selenium. *J. Chron. Diseases* 23: 227-243, 1970
3. T. R. Shearer: Developmental and Postdevelopmental Uptake of Dietary Organic and Inorganic Selenium into the Molar Teeth of Rats. *J. Nutrition* 105: 338-347, 1975
4. T. R. Shearer and D. M. Hadjimarkos: Comparative Distribution of ⁷⁵Se in the Hard and Soft Tissues of Mother Rats and Their Pups. *J. Nutrition* 103: 553-559, 1973
5. D. M. Hadjimarkos: Effect of Selenium on Dental Caries. *Arch. Environ. Health* 10: 893-899, 1965
6. G. Tank and C. A. Storvick: Effect of Naturally Occurring Selenium and Vanadium on Dental Caries. *J. Dent. Res.* 39: 473-488, 1960
7. T. G. Ludwig and B. G. Bibby: Geographic Variations in the Prevalence of Dental Caries in the United States of America. *Caries Res.* 3: 32-43, 1969
8. B. P. Suchkov, I. M. Katsan, and A. I. Gulgasenko: A Study of the Influence of Selenium on the Dental Caries of the Population of the Chernovitsi Region. *Stomatologia* (Moscow) 52: 21-2, 1973
9. W. H. Bowen: The Effects of Selenium and Vanadium on Caries Activity in Monkeys (*M. irus*). *J. Irish Dent. Assn.* 18: 83-88, 1972

NUTRITION CLASSICS



THE
AMERICAN JOURNAL
OF
PHYSIOLOGY

VOLUME XIII: 117-138, 1905

A THEORY OF PROTEIN METABOLISM.

By OTTO FOLIN:

*[From the Chemical Laboratory of the McLean Hospital for the Insane,
Waverley, Massachusetts.]*

IN the preceding paper¹ on "Laws Governing the Composition of Urine,"² it was shown that urine obtained from normal persons does not necessarily exhibit any such constancy in composition as has been supposed to be the case. The analytical results recorded in that paper prove that quantitative changes in the daily protein katabolism are accompanied by pronounced changes in the distribution of the urinary nitrogen and sulphur, and that the variations occur according to laws that can be formulated with a fair degree of precision.

• • •

We have at present two fundamentally different theories concerning the nature of protein metabolism, namely, that of Pflüger and that of Voit. The theory of Pflüger is essentially a modification of an earlier one advanced by Liebig, and is very old. The theory of Voit was first formulated in 1867, after the original theory of Liebig had become untenable, and for a long time Voit's theory enjoyed almost universal acceptance, although it, too, had to be modified in order to be consistent with the facts brought out by Pflüger. Since 1893 Voit's theory may be said to have lost ground. In that year Pflüger¹ published an exceedingly searching criticism of nearly all the facts which Voit had advanced in favor of his theory, and showed that they were either erroneous or capable of a different interpretation. This criticism, accompanied as it was by the experiments of Schöndorff, has never been refuted.

The two theories are briefly as follows: According to Voit, the protein of the absorbed food passes through the blood to the different tissues and cells, and is there katabolized under the influence of the living protoplasm, but without first becoming an integral part of the latter. Voit's fundamental conception seems to me to be that the living protoplasm is in a state of suspension, the "circulating protein" is in solution, and the chemical decompositions that constitute protein katabolism take place only in solution. The small amount of living protoplasm which dies in the course of twenty-four hours is at first only dissolved, thereby becoming a part of the circulating protein derived directly from the food.

Pflüger, on the other hand, believes that there is a very decided chemical difference between circulating protein and living protoplasm. The former is comparatively stable toward oxidizing reagents, while the latter is in a very unstable equilibrium and is particularly susceptible to oxidation. All the protein katabolized is first transformed into bioplasm, becomes an integral part of the living tissue, and only as such undergoes the oxidation that is supposed to constitute the most fundamental chemical decomposition of protein katabolism.

• • •

We have seen from the tables that the composition of urine, representing 15 gm. of nitrogen, or about 95 gm. of protein, differs very widely from the composition of urine representing only 3 gm. or 4 gm. of nitrogen, and that there is a gradual and regular transition from the one to the other. To explain such changes in the composition of the urine on the basis of protein katabolism, we are forced, it seems to me, to assume that katabolism is not all of one kind. There must be at least two kinds. Moreover, from the nature of the changes in the distribution of the urinary constituents, it can be affirmed, I think, that the two forms of protein katabolism are essentially independent and quite different. One kind is extremely variable in quantity, the other tends to remain constant. The one kind yields chiefly urea and inorganic sulphates, no kreatinin, and probably no neutral sulphur. The other, the constant katabolism, is largely represented by kreatinin and neutral sulphur, and to a less extent by uric acid and ethereal sulphates. The more the total katabolism is reduced, the more prominent becomes these representatives of the constant katabolism, the less prominent become the two chief representatives of the variable katabolism.

The fact that the urea and inorganic sulphates represent chiefly the variable katabolism does not preclude the possibility that they also represent to some extent the constant katabolism; but I have reason to believe that it is possible to plan feeding experiments which will yield urines containing very much smaller per cents of these two constituents than I have yet obtained. We know from the experiments of Siven that it is possible to reduce the total protein katabolism still more, and I am confident that in such cases the per cent of urea-nitrogen will sink still lower, and that the nitrogen of the other constituents, particularly of the kreatinin, will again show a corresponding increase.

If there are two distinct forms of protein metabolism represented by two different sets of waste products, it becomes an exceedingly interesting and important problem to determine, if possible, the nature and significance of each. The fact that the kreatinin elimination is not diminished when practically no protein is furnished with the food, and that the elimination of some of the other constituents is only a little reduced under such conditions, shows why a certain amount of protein must be furnished with the food if nitrogen equilibrium is to be maintained. It is clear that the metabolic processes resulting in the end products which tend to be constant in quantity appear to be indispensable for the continuation of life; or, to be more definite, those metabolic processes probably constitute an essential part of the activity which distinguishes living cells from dead ones. I would therefore call the protein metabolism which tends to be constant, *tissue* metabolism or *endogenous* metabolism, and the other, the variable protein metabolism, I would call the *exogenous* or intermediate metabolism.¹

The endogenous metabolism sets a limit to the lowest level of nitrogen equilibrium attainable. Just where that level is fixed will depend on how much, if any, urea is derived from the same katabolic processes that produce the kreatinin. If this can be determined, we shall have a formula expressing more or less definitely the point of lowest attainable protein katabolism, because at such a point the percentage composition of the urine should be practically constant.

The total nitrogen eliminated when this constant composition of the urine has been reached will indicate the lowest attainable level of nitrogen equilibrium. Whether or no such a level can actually be attained, or whether a certain amount of protein must not always fall prey to the exogenous metabolism, can only be settled by a great deal of experimental work.

IRON AND THE REGULATION OF ERYTHROPOIESIS

Recent work is reviewed suggesting that iron deficiency in suckling rabbits is contributory to early postnatal anemia. The evidence against a similar factor operating in humans is discussed.

Key Words: suckling rabbits, erythropoiesis, erythropoietin, anemia, iron therapy

A fall in hemoglobin in the early postnatal period is a widespread phenomenon among mammals, including man. In a study of 25 babies the mean hemoglobin levels fell to less than 12 g per 100 ml by the end of the second month. This was also accompanied by low numbers of erythroid precursors in the marrow.¹

Several explanations have been offered to account for this. First, that oxygen delivery to the tissues stays unimpaired since the oxygen dissociation curve of hemoglobin is shifted to the right,² and for a given oxygen tension, more oxygen is delivered to the tissues. The major substances present in the red cell causing such a shift is 2, 3 diphosphoglycerate (2, 3 DPG). This substance has been documented as being increased in early postnatal life in man² and in other animals.³ It must be said, however, that this may be putting the cart before the horse, since anemia per se can increase intracellular 2, 3 DPG, presumably as a compensatory mechanism, as can hypoxia from other causes.

The second possible explanation of this 'early anemia' is immaturity of the mechanisms concerned. This could be immaturity of the organs producing the erythrocyte-stimulating factor (ESF), of the marrow itself, or of the receptors which provoke ESF production by detecting hypoxia. That is, there may be an alteration in the threshold level required to trigger ESF production.

Third, anemia during this period could be due to a lack of some essential requirement for red cell production due to the limited diet ingested, i.e., milk, which is particularly low in iron content.

Anemia is more severe in premature infants who start life with lower body iron stores.⁴ Supplementation of the diet with iron prevents the development of later anemia in these infants and in term infants.⁵ However, there is less evidence of an effect of iron on the so-called 'physiological anemia' of early postnatal life.

Experimental proof can most easily be sought in animals. The Halvorsens chose the rabbit for a study on ESF levels in suckling rabbits.⁶ Baby rabbits were studied in the interval between birth and weaning at 20 days. They were the offspring of mothers whose diet had been replete in iron for at least the last week of pregnancy. Normal hematological parameters, i.e., hemoglobin, hematocrit, and reticulocyte counts were studied under varying conditions. The volume of red cells was measured by a standard ⁵¹Cr labeling technique. Plasma ESF was assessed by measuring the stimulatory effect of plasma samples on the incorporation of ⁵⁹Fe into red cells in mice whose innate erythropoiesis had been suppressed by anoxia-induced polycythemia.

Regular intramuscular injections of iron in eight baby rabbits from day ten prevented the fall in hemoglobin that was maximal by day 20 in a control group of untreated rabbits. At this time the hemoglobin and hematocrit were 8.9 g percent

and 30.4 percent on the average respectively in the latter group. In the iron-treated group, the same parameters were 12.6 g percent and 48 percent. There was no significant difference in the mean corpuscular hemoglobin concentrations between the two groups. The average volume of red cells on day 20 was 19 ml per kilogram in the control group and 31 ml per kilogram in the iron-treated group.

ESF levels in the control rabbits were low until day 20 and then rose when the diet changed at weaning. Thus, although anemia develops over this period, there appears to be no compensatory stimulus for increased erythropoiesis. That animals of this age are capable of mounting such a response was shown by exposing them to hypoxia. In the first two days of life no rise could be elicited but between day three and day 20, the ESF rose to levels comparable to those attained by adult rabbits exposed to hypoxia.

These experiments were extended to show the effect of additional iron supplementation. In spite of the marked rise in ESF, hypoxia without iron supplementation had no great effect on increasing the volume of red cells. If, however, iron therapy was also given, the average volume was 40 ml per kilogram by day 20 compared with 23 ml per kilogram for a group made hypoxic but not given iron.

Blood loss also resulted in a rise of ESF values both at ten and 18 days of age. For example, an 18-day old rabbit with ESF levels producing 4.34 percent incorporation of a dose of ^{59}Fe , attained an increase to 14.24 percent incorporation 18 hours after removal of 35 percent of its red cell volume. Thus, directly and indirectly induced tissue hypoxia can result in a rise in ESF at this age.

It seems, therefore, that at least in the rabbit, early anemia is in part due to iron lack, and is amenable to iron therapy. This is perhaps an unexpected result since there is evidence that in untreated rabbits there are plenty of iron stores in the liver at the end of the suckling period.⁷ Presumably these iron stores are not labile and not readily available.

Caution must be exercised in extrapolating these conclusions to other species, particularly to human babies. Iron supplementation from birth does not appear to prevent the drop of hemoglobin in the first six weeks in man.⁸ In the first months of life iron stores progressively accumulate⁹ and much of this iron is derived from the catabolism of red cells responsible for the high hemoglobin levels at birth. In the first few months the developing anemia is normochromic and not until about six months may it become hypochromic as rapid growth exhausts the iron stores. This is the stage in humans which can be obviated by supplementation with dietary iron. Erythropoietin is not usually detectable in plasma between the second and 60th days of life.¹⁰ The human infant, like the rabbit is, however, capable of mounting a response to hypoxia since higher levels are found in cyanotic congenital heart disease.¹¹ It seems that in man this early anemia is truly physiological, and accompanied by delivery of a constant amount of oxygen to the tissues. In the rabbit, the situation is apparently less straightforward. □

1. D. G. Gairdner, J. Marks, and J. D. Roscoe: Blood Formation in Infancy Part II Normal Erythropoiesis. *Arch. Dis. Child.* 27: 214-221, 1952
2. M. Delivoria-Papadopoulos, N. P. Roncevic, and F. A. Oski: Postnatal Changes in Oxygen Transport of Term, Premature, and Sick Infants: The Role of Red Cell 2-3-Diphosphoglycerate and Adult Hemoglobin. *Pediat. Res.* 5: 235-245, 1971
3. M. H. Blunt, J. L. Kitchen, S. M. Mayson, and T. H. J. Huisman: Red Cell 2-3-Diphosphoglycerate and Oxygen Affinity in Newborn Goats and Sheep. *Proc. Soc. Exp. Biol. Med.* 138: 800-812, 1971
4. M. K. Gorten and E. R. Cross: Iron Metabolism of Premature Infants: II. Prevention of Iron Deficiency. *J. Pediat.* 64: 509-520, 1964
5. M. B. Andelman and B. R. Sered: Utilization of Dietary Iron by Term Infants. A Study of 1,048 Infants from a Low Socioeconomic Population. *Am. J. Dis. Child.* 111: 45-55, 1966

6. K. Halvorsen and S. Halvorsen: The Regulation of Erythropoiesis in the Suckling Rabbit. *Pediat. Res.* 8: 176-183, 1974
7. K. Halvorsen and S. Halvorsen: The 'Early Anemia'; Its Relation to Postnatal Growth Rate, Milk Feeding, and Iron Availability Experimental Study in Rabbits. *Arch. Dis. Child.* 48: 842-849, 1973
8. A. Marsh, H. Long, and E. Stierwalt: Comparative Hematologic Response to Iron Fortification of a Milk Formula for Infants. *Pediatrics* 24: 404-412, 1959
9. F. A. Langley: Haemopoiesis and Siderosis in Foetus and Newborn. *Arch. Dis. Child.* 26: 64-75, 1951
10. D. L. Mann, M. D. Sites, R. M. Donati, and M. I. Gallagher: Erythropoietic Stimulating Activity during the First Ninety Days of Life. *Proc. Soc. Exp. Biol. Med.* 118: 212-214, 1965
11. S. Halvorsen: Plasma Erythropoietin Levels in Cord Blood and in Blood during the First Weeks of Life. *Acta Pediat.* (Stockholm) 52: 425-435, 1963

New

Unique

INDEX OF NUTRITION EDUCATION MATERIALS

The Index of Nutrition Education Materials has been prepared by the Office of Education of the Nutrition Foundation to assist teachers and the general public in acquiring useful teaching aids and educational materials.

The Index lists more than 1,000 booklets, pamphlets, audio-visual aids and lists of recommended books available from federal agencies, professional societies, and educational organizations or foundations. Other sources for education material are included.

Gives addresses of important local contacts and sources in each state.

Source, subject matter, readership level and description are given for each publication in color-coded sections for easy reference. \$5.00 postpaid

Order from:

OFFICE OF EDUCATION
THE NUTRITION FOUNDATION
888 Seventeenth Street, N.W.
Washington, D. C. 20006

FAO/WHO HANDBOOK ON HUMAN NUTRITIONAL REQUIREMENTS, 1974

Over the past twenty years, FAO/WHO have jointly convened eight meetings of expert groups which have reported on requirements for energy,¹ protein,¹ vitamin A,² thiamine,² riboflavin,² niacin,² ascorbic acid,³ vitamin D,³ vitamin B₁₂,³ folates,³ iron,³ and calcium.⁴ In addition, a WHO Expert Committee on Trace Elements in Human Nutrition reviewed the evidence concerning human needs for trace elements other than iron, iodine and fluorine.⁵ The reports deal with various aspects of the individual nutrients including biochemistry, physiology, evidence for roles in clinical medicine, the epidemiology of known deficiencies, and in some instances the ecology of man in relation to the food supply.

A composite summary and commentary which deals with the total spectrum of these nutrients has been recently published by FAO and WHO⁶ and is intended for the use of applied nutritionists, agricultural planners and food administrators, as well as teachers in colleges and secondary schools and those concerned with health education. The correlation of reports and drafting of the handbook was undertaken by Dr. R. Passmore, Mrs. D. L. Bocobo, Dr. B. M. Nicol, Dr. M. Narayana Rao, Dr. G. H. Beaton and Dr. E. M. DeMayer.

Energy

Carbohydrates, fats, proteins and alcohol are recognized energy sources. The consideration of energy requirements is presented in both kilocalories (kcal) and in joules. These units are defined as: a kilocalorie is the amount of heat required to raise the temperature of a liter of water from fifteen degrees centigrade to sixteen degrees centigrade; one joule is equal to the energy expended when one kilogram is moved through one meter by one newton

(a force which accelerates one kilogram by 1 m/sec²). The report states:

"One kilocalorie is equal to 4.184 kilojoules (kJ). The energy content of diets and the energy requirements of humans usually exceed 1 000 kJ and are generally expressed in terms of megajoules (MJ).

"The following factors may be used for converting calories to joules and vice versa:

1 kcal = 4.184 kJ

1 000 kcal = 4 184 kJ

1 000 kcal = 4.184 MJ

1 kJ = 0.239 kcal

1 000 kJ = 239 kcal

1 MJ = 239 kcal

"The approximate energy values of the body fuels are the following: for carbohydrate, 4 kcal or 16.7 kJ per gram; for fat, 9 kcal or 37.7 kJ per gram; for protein, 4 kcal or 16.7 kJ per gram; and for alcohol, 7 kcal or 29.3 kJ per gram. These are net values, allowing for the small losses of energy in the faeces and also for the energy lost in the urine in the form of urea and other nitrogenous end-products of protein metabolism which cannot be completely broken up in the body. . . .

"The subject of the metabolism of alcohol in humans and laboratory animals has been investigated from time to time. The purpose of these studies has been to determine whether alcohol may serve the same purpose in the energy economy as ordinary carbohydrate does in saving protein and in providing energy for muscular activity, the deposition of fat, and the generation of heat to maintain body temperature.

"It has been observed that under conditions of moderate intake most of the potential energy of the ingested alcohol is available for muscular work and for the production of body heat. The partial replacement of carbohydrate or fat in the diet by an amount of alcohol equal in energy content has also been shown to be effective in the synthesis of body tissue.

"The body can oxidize alcohol at a limited rate. A healthy, well-fed adult who in terms of body weight consumes alcohol in quantities of less than 2 g/kg in twenty-four hours oxidizes it at a constant but limited rate of about 100 mg/kg per hour. A 65-kg man and a 55-kg woman can thus obtain, respectively, 700 kcal (2.9 MJ) and 525 kcal (2.2 MJ) daily from alcohol."

The energy expenditure of adults has been estimated for a variety of occupations (Tables 1 and 2). The influence of body size and composition, age, and climate is briefly considered. The requirements during pregnancy and lactation are estimated (Table 4), as are the requirements of infants and children.

Table 1
Energy Expenditure of a 65-kg Reference Man
Distributed over 24 Hours and Effect of Occupation

Distribution of activity	Light activity		Moderately active		Very active		Exceptionally active	
	kilo-calories	mega-joules	kilo-calories	mega-joules	kilo-calories	mega-joules	kilo-calories	mega-joules
In bed (8 hours)	500	2.1	500	2.1	500	2.1	500	2.1
At work (8 hours)	1 100	4.6	1 400	5.8	1 900	8.0	2 400	10.0
Nonoccupational activities (8 hours)	700-1 500	3.0-6.3	700-1 500	3.0-6.3	700-1 500	3.0-6.3	700-1 500	3.0-6.3
Range of energy activities (24 hours)	2 300-3 100	9.7-13.0	2 600-3 400	10.9-14.2	3 100-3 900	13.0-16.3	3 600-4 400	15.1-18.4
Mean (24 hours)	2 700	11.3	3 000	12.5	3 500	14.6	4 000	16.7
Mean (per kg of body weight)	42	0.17	46	0.19	54	0.23	62	0.26

Table 2
Energy Expenditure of a 55-kg Reference Woman
Distributed over 24 Hours and Effect of Occupation

Distribution of activity	Light activity		Moderately active		Very active		Exceptionally active	
	kilo-calories	mega-joules	kilo-calories	mega-joules	kilo-calories	mega-joules	kilo-calories	mega-joules
In bed (8 hours)	420	1.8	420	1.8	420	1.8	420	1.8
At work (8 hours)	800	3.3	1 000	4.2	1 400	5.9	1 800	7.5
Nonoccupational activities (8 hours)	580-980	2.4-4.1	580-980	2.4-4.1	580-980	2.4-4.1	580-980	2.4-4.1
Range of energy expenditure (24 hours)	1 800-2 200	7.5-9.2	2 000-2 400	8.4-10.1	2 400-2 700	10.1-11.8	2 800-3 200	11.7-13.4
Mean (24 hours)	2 000	8.4	2 200	9.2	2 600	10.9	3 000	12.5
Mean (per kg of body weight)	36	0.15	40	0.17	47	0.20	55	0.23

Protein

The protein requirements are based upon the most recent (1971) Joint FAO/WHO Expert Group on Energy and Protein Requirements¹ which considered data obtained by both the "factorial" approach as well as nitrogen balance studies. A figure of 0.57 grams and 0.52 grams per day per kilogram of body weight as a safe level of protein intake in terms of cow's milk or egg protein for an adult man or woman was agreed upon. The safe level of intake is intended to be the amount "necessary to meet the physiological needs and maintain the health of nearly all individuals in the group and is therefore higher than the average protein requirement." The nutritive value of mixed dietary proteins, generally lower than that of milk or egg proteins, is assessed by introducing a correction for protein quality in keeping with the formula shown in Figure 1.

The handbook notes that "a strong warning should be sounded against the condemnation of a food because, when eaten alone, its proteins do not have a high biological value" and that "practical nutrition is concerned with the nutritive value of diets not of individual foods."

The safe levels of protein intake when energy requirements are fully met are stated to be 37 grams per day for a reference man weighing 65 kilograms and 25 grams per day for a reference woman weighing 55 kilograms. Persons engaged in heavy manual work, where energy needs are greater, normally increase the intake of protein, but no satisfactory evidence of an increased protein need resulting from greater physical activity per se exists. The effects of infestations and infections are noted, but no quantitative estimates are attempted. The daily protein requirements per kilogram of body weight for a child during the first year of life are shown in Table 3.

Table 3 — The daily protein requirements per kilogram of body weight of a child during the first year of life.

Months	Grams*
< 3	2.40
3-6	1.85
6-9	1.62
9-11	1.44

* In terms of milk or egg protein.

Vitamins

The relatively brief discussions of vitamins are intended to familiarize the non-technical reader with the nutritional role of the substances rather than elaborate upon the basis of dietary requirements. An exception to this is the explanation that 60 mg of tryptophan is required to produce one mg of niacin and hence by definition, a niacin equivalent is equal to one mg of niacin or 60 mg of tryptophan.

Iron

The discussion of iron is more extensive. Key factors taken into account in estimating the requirements include the total daily loss of 0.9 mg of iron in an adult man weighing 65 kilograms. Absorption ranges from 10 to 30 percent of food iron, but for estimating absorption or iron in diets a weighted upper value of 20% has been taken as the percentage of iron absorbed from foods of animal and soybean origin. The contribution made by iron deficiency anemia to weakness, ill health and sub-standard performance of millions of persons throughout the world, and the estimates of intakes of iron are commented upon in part as follows:

"Normal mixed human diets of good quality contain approximately 12-15 mg of iron, of which slightly more than 1 mg is absorbed. This amount is adequate for adult males, but it is inadequate for adolescent girls or women on diets of less than 10 percent calorie content from

Figure 1:

Requirement of dietary protein

=

safe level of protein intake x protein value of egg

protein value of dietary protein

animal foods; for this reason the iron requirements for the latter have been set by the FAO/WHO Expert Committee at 24 and 28 mg per day, respectively. Obviously it is difficult to design a diet which normally contains this amount of iron, and it is to be expected that under these circumstances a certain proportion of adolescent girls and menstruating women will not be able to meet their requirement without recourse to iron supplementation.

"In certain affluent countries, notably in the U.S.A., where populations consume highly refined foods, normal diets have been found to contain 6-7 mg of iron, a level which is not high enough to satisfy the requirements. The iron levels in such diets can be improved by fortification. The availability of iron added to foods is affected by the form of iron added, the nature of the vehicle, and the quality of other dietary constituents. Most of the evidence suggests that ferrous sulphate, now being used in certain fortification programmes, is among the most available forms."

For the reader interested in the iron requirements for pregnancy and lactation, the Joint FAO/WHO Expert Committee report on nutrition in pregnancy and lactation can serve as a valuable reference.

Iodine

The use of iodized salt is regarded as the most successful and most widely adopted method for enhancing dietary iodine. A level of 0.01 percent potassium iodide or iodate or commercially iodized salt is advocated. Based upon an estimated average adult use of 6 to 7 grams of salt daily, the resulting iodine intake would amount to 0.48 mg.

Fluoride

The fluoridation of water supplies to bring the concentration of fluoride to 1 ppm is advocated as a safe, economical and efficient way to reduce tooth decay, despite the fact that "the subject is very liable to produce intense outbursts of irrational emotion at the local government level in many developed countries." The "average daily diet" is calculated to provide 0.25 to 0.35 mg of fluoride with an additional

average adult ingestion of 1.0 to 1.5 mg daily from drinking and cooking water that contains 1 ppm of fluoride. For children 1 to 12 years old, water may contribute anywhere from 0.4 to 1.1 mg of fluoride per day.

For those other trace elements essential for human nutrition, but not tabulated in Table 1, the handbook treats each as follows:

Zinc

"The zinc requirement for an adult male, derived factorially, is 2.2 mg per day. The amounts of dietary zinc needed to meet requirements vary with the composition of the diet and the availability of the element. If the availability of Zn is 10 percent, the amount of dietary zinc needed daily to meet the requirement would be 22 mg. Growing children and pregnant and lactating women need more.

"Animal foodstuffs are dependable sources of zinc. Beef, pork, and lamb may contain 20-60 $\mu\text{g/g}$, and milk 3-5 $\mu\text{g/g}$. Fish and other sea foods contain more than 15 $\mu\text{g/g}$. Whole cereals are also rich sources of zinc, but appreciable amounts are lost during milling."

Magnesium

"Adult magnesium requirements have been estimated to lie between 200 and 300 mg per day. An intake of 300 mg daily has been found to maintain a positive balance in women. Estimates of magnesium requirements are based on extremely limited information regarding the absorption, metabolism, and excretion of this nutrient; accordingly, the allowances proposed must be regarded as provisional.

"Magnesium is widely distributed in plants. Meat and viscera are rich sources of the element. Milk is a relatively poor source. In a mixed diet containing abundant animal products, magnesium appears to be 30-40 percent available."

Copper

"A realistic allowance of copper for infants and young children, incorporating a desirable margin of safety, is 80 $\mu\text{g/kg}$ per day. It is probable that 40 μg per kilogram of body weight per day is adequate

for older children, and that approximately 30 μg per kilogram daily is sufficient for adults.

"Copper is widely distributed in foodstuffs, and a diet of even mediocre quality contains 2-3 mg per day, enough to meet the requirements of man. The richest sources of dietary copper are liver, kidney, shellfish, nuts, raisins, and dried legumes. Milk is a poor source of the element. Homogenized cow's milk contains from 0.015 to 1.18 mg of copper per litre, much less on the average than the 0.6 to 1.05 mg present in a litre of human milk."

Chromium

The handbook states that there is some evidence that chromium is an essential nutrient for man and comments that:

"Absorption of trivalent chromium in man, just as in the rat, may vary from less than 1 percent to 10 or 25 percent. The availability of chromium depends upon its chemical nature in foodstuffs. Dietary intakes varying from 20-50 mg per day are required to compensate for the loss of the element through the urine.

"Chromium is present in lower concentrations in vegetable foods than it is in animal foods. Intakes of the element can vary significantly, from 5 to over 100 mg daily. It is present in significant amounts in drinking water."

Selenium, Cobalt and Molybdenum

The need for selenium by a variety of laboratory and farm animals is documented, but sufficient evidence is not available for the establishment of human requirements. Similarly, man has a requirement for a cobalt containing vitamin B_{12} , but except for this there is no known human requirement for the element.

Similarly, there is no evidence of any clinical manifestation of deficiency molybdenum in man. On the other hand, studies in human adults have shown

". . . that molybdenum equilibrium or a slight positive balance may be main-

tained if the diet provides 2 μg molybdenum per kilogram of body weight daily. This figure can be tentatively suggested as the requirement for humans."

The handbook concludes that the recommended intake of nutrients (Table 4) is based on contemporary nutritional science and that while newer knowledge may allow some of the recommendations to be fixed more precisely, it is unlikely that major changes will be required. These figures are recommended by the agencies to be used as practical guides for agricultural planning in all countries.

It is of interest to compare these requirements with the recommended dietary allowances issued in the United States in 1974. These are summarized for easy reference in Table 5. The Canadian recommended daily nutrient intakes are summarized in Table 6.

Copies of the handbook are available for \$1.00 from UNIPUB, Inc., 650 First Avenue, P. O. Box 433, Murray Hill Station, New York, New York 10016. □

1. *Energy and Protein Requirements*. Report of a Joint FAO/WHO Expert Group, FAO, Rome, 1972
2. *Requirements of Vitamin A, Thiamine, Riboflavin and Niacin*. Report of a Joint FAO/WHO Expert Group, FAO, Rome, 1965
3. *Requirements of Ascorbic Acid, Vitamin D, Vitamin B_{12} , Folate and Iron*. Report of a Joint FAO/WHO Expert Group, FAO, Rome, 1970
4. *Calcium Requirements*. Report of a FAO/WHO Expert Group, FAO, Rome, 1961
5. *Other Trace Elements Essential for Human Nutrition*. Discussed by the WHO Expert Committee on Trace Elements in Human Nutrition in 1973. WHO Technical Report Series, No. 532, 1973
6. *Handbook on Human Nutritional Requirements*. Published by FAO and WHO. FAO Nutritional Studies No. 28; WHO Monograph Series No. 61, Rome, 1974

Table 4 — Recommendations

Age	Body weight	Energy (1)		Protein (1,2)
		kilo-calories	mega-joules	grams
Children				
<1	7.3	820	3.4	14
1-3	13.4	1 360	5.7	16
4-6	20.2	1 830	7.6	20
7-9	28.1	2 190	9.2	25
Male adolescents				
10-12.	36.9	2 600	10.9	30
13-15.	51.3	2 900	12.1	37
16-19.	62.9	3 070	12.8	38
Female adolescents				
10-12.	38.0	2 350	9.8	29
13-15.	49.9	2 490	10.4	31
16-19.	54.4	2 310	9.7	30
Adult man				
(moderately active)	65.0	3 000	12.6	37
Adult woman				
(moderately active)	55.0	2 200	9.2	29
Pregnancy				
(later half)		+ 350	+ 1.5	38
Lactation				
(first 6 months)		+ 550	+ 2.3	46

¹Energy and Protein Requirements. Report of a Joint FAO/WHO Expert Group, FAO, Rome, 1972. — ²As egg or milk protein. — ³Requirements of Vitamin A, Thiamine, Riboflavin and Niacin. Report of a Joint FAO/WHO Expert Group, FAO, Rome, 1965. — ⁴As retinol. — ⁵Requirements of Ascorbic Acid, Vitamin D, Vitamin B₁₂, Folate and Iron. Report of a Joint FAO/WHO Expert Group, FAO, Rome, 1970. — ⁶As cholecalciferol. — ⁷Calcium Requirements. Report of a FAO/WHO Expert Group, FAO, Rome, 1961. — ⁸On each line the lower value applies when over 25 percent of calories in the diet come from

Recommended Intakes of Nutrients (FAO/WHO)

Vitamin A (3,4)	Vitamin D (5,6)	Thiamine (3)	Riboflavin (3)	Niacin (3)	Folic acid (5)	Vitamin B ₁₂ (5)	Ascorbic acid (5)	Calcium (7)	Iron (5,8)
<i>micro-grams</i>	<i>micro-grams</i>	<i>milli-grams</i>	<i>milli-grams</i>	<i>milli-grams</i>	<i>micro-grams</i>	<i>micro-grams</i>	<i>milli-grams</i>	<i>grams</i>	<i>milli-grams</i>
300	10.0	0.3	0.5	5.4	60	0.3	20	0.5-0.6	5-10
250	10.0	0.5	0.8	9.0	100	0.9	20	0.4-0.5	5-10
300	10.0	0.7	1.1	12.1	100	1.5	20	0.4-0.5	5-10
400	2.5	0.9	1.3	14.5	100	1.5	20	0.4-0.5	5-10
575	2.5	1.0	1.6	17.2	100	2.0	20	0.6-0.7	5-10
725	2.5	1.2	1.7	19.1	200	2.0	30	0.6-0.7	9-18
750	2.5	1.2	1.8	20.3	200	2.0	30	0.5-0.6	5-9
575	2.5	0.9	1.4	15.5	100	2.0	20	0.6-0.7	5-10
725	2.5	1.0	1.5	16.4	200	2.0	30	0.6-0.7	12-24
750	2.5	0.9	1.4	15.2	200	2.0	30	0.5-0.6	14-28
750	2.5	1.2	1.8	19.8	200	2.0	30	0.4-0.5	5-9
750	2.5	0.9	1.3	14.5	200	2.0	30	0.4-0.5	14-28
750	10.0	+0.1	+0.2	+2.3	400	3.0	30	1.0-1.2	(9)
1 200	10.0	+0.2	+0.4	+3.7	300	2.5	30	1.0-1.2	(9)

animal foods, and the higher value when animal foods represent less than 10 percent of calories. — ⁹For women whose iron intake throughout life has been at the level recommended in this table, the daily intake of iron during pregnancy and lactation should be the same as that recommended for nonpregnant, nonlactating women of childbearing age. For women whose iron status is not satisfactory at the beginning of pregnancy, the requirement is increased, and in the extreme situation of women with no iron stores, the requirement can probably not be met without supplementation.

TABLE 5 — RECOMMENDED DAILY DIETARY ALLOWANCES.¹ Revised 1973
FOOD AND NUTRITION BOARD, NATIONAL ACADEMY OF SCIENCES-NATIONAL RESEARCH COUNCIL
Designed for the maintenance of good nutrition of practically all healthy people in the U.S.A.

	Fat-Soluble Vitamins										Water-Soluble Vitamins								Minerals					
	(years) From Up to	Weight (kg)	Weight (lbs)	Height (cm)	Height (in)	Energy (kcal) ²	Protein (g)	Vitamin A Activity (RE) ³	Vitamin A Activity (IU)	Vita- min D (IU)	Vita- min E Activity ⁵ (IU)	Ascor- bic Acid (mg)	Fola- cin ⁶ (μg)	Nia- cin ⁷ (mg)	Ribo- flavin (mg)	Thia- min (mg)	Vita- min B ₆ (mg)	Vita- min B ₁₂ (μg)	Cal- cium (mg)	Phos- phorus (mg)	Iodine (μg)	Iron (mg)	Mag- nesium (mg)	Zinc (mg)
Infants	0.0-0.5	6	14	60	24	kg x 117	kg x 2.2	420 ⁴	1,400	400	4	35	50	5	0.4	0.3	0.3	0.3	360	240	35	10	60	3
	0.5-1.0	9	20	71	28	kg x 108	kg x 2.0	400	2,000	400	5	35	50	8	0.6	0.5	0.4	0.3	540	400	45	15	70	5
Children	1-3	13	28	86	34	1300	23	400	2,000	400	7	40	100	9	0.8	0.7	0.6	1.0	800	800	60	15	150	10
	4-6	20	44	110	44	1800	30	500	2,500	400	9	40	200	12	1.1	0.9	0.9	1.5	800	800	80	10	200	10
	7-10	30	66	135	54	2400	36	700	3,300	400	10	40	300	16	1.2	1.2	1.2	2.0	800	800	110	10	250	10
Males	11-14	44	97	158	63	2800	44	1,000	5,000	400	12	45	400	18	1.5	1.4	1.6	3.0	1200	1200	130	18	350	15
	15-18	61	134	172	69	3000	54	1,000	5,000	400	15	45	400	20	1.8	1.5	2.0	2.0	1200	1200	150	18	400	15
	19-22	67	147	172	69	3000	52	1,000	5,000	400	15	45	400	20	1.8	1.5	2.0	3.0	800	800	140	10	350	15
	23-50	70	154	172	69	2700	56	1,000	5,000		15	45	400	18	1.6	1.4	2.0	3.0	800	800	130	10	350	15
	51 +	70	154	172	69	2400	56	1,000	5,000		15	45	400	16	1.5	1.2	2.0	3.0	800	800	110	10	350	15
Females	11-14	44	97	155	62	2400	44	800	4,000	400	12	45	400	16	1.3	1.2	1.6	3.0	1200	1200	115	18	300	15
	15-18	54	119	162	65	2100	48	800	4,000	400	12	45	400	14	1.4	1.1	2.0	3.0	1200	1200	115	18	300	15
	19-22	58	128	162	65	2100	46	800	4,000	400	12	45	400	14	1.4	1.1	2.0	3.0	800	800	100	18	300	15
	23-50	58	128	162	65	2000	46	800	4,000		12	45	400	13	1.2	1.0	2.0	3.0	800	800	100	18	300	15
	51 +	58	128	162	65	1800	46	800	4,000		12	45	400	12	1.1	1.0	2.0	3.0	800	800	80	10	300	15
Pregnant						+300	+ 30	1,000	5,000	400	15	60	800	+2	+0.3	+0.3	2.5	4.0	1200	1200	125	18+ ⁸	450	20
Lactating						+500	+ 20	1,200	6,000	400	15	80	600	+4	+0.5	+0.3	2.5	4.0	1200	1200	150	16	450	25

¹The allowances are intended to provide for individual variations among most normal persons as they live in the United States under usual environmental stresses. Diets should be based on a variety of common foods in order to provide other nutrients for which human requirements have been less well defined. See text for more-detailed discussion of allowances and of nutrients not tabulated.

²Kilojoules (KJ) = 4.2 x kcal.

³Retinol equivalents.

⁴Assumed to be all as retinol in milk during the first six months of life. All subsequent intakes are assumed to be one-half as retinol and one-half as β-carotene when calculated from international units. As retinol equivalents, three-fourths are as retinol and one fourth as β-carotene.

⁵Total vitamin E activity, estimated to be 80 percent as α-tocopherol and 20 percent other tocopherols. See text for variation in allowances.

⁶The folacin allowances refer to dietary sources as determined by *Lactobacillus casei* assay. Pure forms of folacin may be effective in doses less than one-fourth of the RDA.

⁷Although allowances are expressed as niacin, it is recognized that on the average 1 mg of niacin is derived from each 60 mg of dietary tryptophan.

⁸This increased requirement cannot be met by ordinary diets; therefore, the use of supplemental iron is recommended.

Age (years)	Sex
0-6 mos.	Both
7-11 mos.	Both
1-3	Both
4-6	Both
7-9	M F
10-12	M F
13-15	M F
16-18	M F
19-35	M F
36-50	M F
51 +	M F
Pregnant Lactating	

Table 6. Recommended Daily Nutrient Intakes (Canada)

Weight (k j)	Height (cm)	Energy (Cal)	Protein (g)	Vit. A ₁ (μg RE)	Vit. D (μg)	Vit. E (mg)	Thiamin (mg)	Niacin (mg)	Riboflavin (mg)	Vit. B ₆ (mg)	Folate (μg)	Vit. B ₁₂ (μg)	Ascorbic Acid (mg)	Calcium (mg)	Phosphorus (mg)	Magnesium (mg)	Iodine (μg)	Iron (mg)	Zinc (mg)
6	—	kg x 117	kg x 2.2(2.0)	400	10	3	0.3	5	0.4	0.3	40	0.3	20	500	250	50	35	7	4
9	—	kg x 108	k x 1.4	400	10	3	0.5	6	0.6	0.4	60	0.3	20	500	400	50	50	7	5
13	90	1400	22	400	10	4	0.7	9	0.8	0.8	100	0.4	20	500	500	75	70	8	5
19	110	1800	27	500	5	5	0.9	12	1.1	1.3	100	1.5	20	500	500	100	90	9	6
27	129	2200	33	700	2.5	6	1.1	14	1.3	1.6	100	1.5	30	700	700	150	110	10	7
27	128	2000	33	700	2.5	6	1.0	13	1.2	1.4	100	1.5	30	700	700	150	100	10	7
36	144	2500	41	800	2.5	7	1.2	17	1.5	1.8	100	3.0	30	900	900	175	130	11	8
38	145	2300	40	800	2.5	7	1.1	15	1.4	1.5	100	3.0	30	1000	1000	200	120	11	9
51	162	2800	52	1000	2.5	9	1.4	19	1.7	2.0	200	3.0	30	1200	1200	250	140	13	10
49	159	2200	43	800	2.5	7	1.1	15	1.4	1.5	200	3.0	30	800	800	250	110	14	10
64	172	3200	54	1000	2.5	10	1.6	21	2.0	2.0	200	3.0	30	1000	1000	300	160	14	12
54	161	2100	43	800	2.5	6	1.1	14	1.3	1.5	200	3.0	30	700	700	250	110	14	11
70	176	3000	56	1000	2.5	9	1.5	20	1.8	2.0	200	3.0	30	800	800	300	150	10	10
56	161	2100	41	800	2.5	6	1.1	14	1.3	1.5	200	3.0	30	700	700	250	110	14	9
70	176	2700	56	1000	2.5	8	1.4	18	1.7	2.0	200	3.0	30	800	800	300	140	10	10
56	161	1900	41	800	2.5	6	1.0	13	1.2	1.5	200	3.0	30	700	700	250	100	14	9
70	176	2300	56	1000	2.5	8	1.4	18	1.7	2.0	200	3.0	30	800	800	300	140	10	10
56	161	1800	41	800	2.5	6	1.0	13	1.2	1.5	200	3.0	30	700	700	250	100	9	9
		+300	+20	+100	+2.5	+1	+0.2	+2	+0.3	+0.5	+ 50	+1.0	+ 20	+500	+500	+25	+15	+1	+3
		+500	+24	+400	+2.5	+2	+0.4	+7	+0.6	+0.6	+ 50	+0.5	+ 30	+500	+500	+75	+25	+1	+7

DEFINITIONS:

Cal — calorie
cm — centimetre, 1 centimetre = .39 inches
g — gram
kg — kilogram, 1 kilogram = 2.2 pounds
mg — milligram
μg — microgram

Source: Health and Welfare Canada. Committee for Revision of the Canadian Dietary Standard, Bureau of Nutritional Sciences. (Revised 1974)

New Fellowships in Nutrition Available for Physicians

Fellowships to provide support for significant periods of sub-specialty training in the field of nutrition are now available to qualified young physicians in both the United States and Canada. Nutrition teaching and research scholarships for a three year period carrying stipends of \$12,000 for the first year, \$13,000 for the second, and \$14,000 for the third year, supported by The Nutrition Foundation, are being awarded by the American College of Physicians.

Similar teaching and research fellowships in the field of nutrition to develop Canadian pediatricians with specialized competence in nutrition are available from the Hospital for Sick Children Foundation, Toronto. The value of these fellowships is \$15,000 annually plus return economy air fare to the location of study. The fellowships may be of one or two years duration.

American College of Physicians Nutrition Teaching and Research Scholarships

Two Teaching and Research scholarships specifically in the field of medical nutrition will be supported by The Nutrition Foundation, Inc.

Each scholar will be appointed for a term of three years with stipends increasing from \$12,000 in the first year to \$13,000 and \$14,000 in the second and third years, respectively. The stipend may be supplemented by the host institution.

It is the intention of the American College of Physicians to select physicians with interests and skills both in teaching and research who show promise of excellent future development based on good undergraduate and postdoctoral training.

During their period of appointment, the Teaching and Research Scholars in the field of Medical Nutrition should predominantly direct their professional activity to problems of nutrition in medicine as related to diseases of nutrition per se or nutritional

aspects of medical diseases in the broad sense.

At the time of an appointment to begin in July of each year, the individual shall have been graduated from medical school no more than eight years. In addition, the candidate shall have no less than four years of professional training after medical school graduation. The applicant must have at least four years of postdoctoral training in medicine. The Scholarships are restricted to citizens of the United States and Canada.

Candidates must be proposed by the Chairman of the Department of Medicine in any institution in the United States or Canada with a recognized program of medical education and research, and endorsed by the Dean or the appropriate signing official of the university or college concerned. Not more than one candidate may be recommended by a Chairman of a Department of Medicine in any given institution in any calendar year.

The Chairman of the Department of Medicine in any such institution shall be responsible for presenting a careful and complete evaluation of the individual he or she wishes to recommend for a Teaching and Research Scholarship on the official form. Notification of the recommendation will be sent to the College Governor of the State or Province concerned by the ACP.

The sponsor must be prepared to provide adequate facilities for the work of the Scholar, or to help him or her obtain such facilities in an institution in which the teaching and research is to be carried out. If the Scholar is to carry on teaching and research in an institution other than the one from which the initial proposal is made, the grant for such work elsewhere will follow the Scholar.

All proposals must reach the Executive Offices of the American College of Phy-

sicians by September 1 of each year. Final selection will be made at the mid-November meeting of the Fellowships and Scholarships Committee and of the Board of Regents.

For information and Official Proposal Form, write: Edward C. Rosenow, Jr., M.D., F.A.C.P., Executive Vice President, American College of Physicians, 4200 Pine Street, Philadelphia, Pennsylvania 19104.

Hospital for Sick Children Foundation Fellowship

The Hospital for Sick Children Foundation is offering a Fellowship in any of the following disciplines: Nutrition, Infectious Diseases, Mental Retardation, Environmental Health, Clinical Pharmacology.

This Fellowship is offered by the Foundation realizing the need for increased facilities in these disciplines in Canada. It is the hope that training at the postdoctoral level will lead to the establishment in Canada of pediatric facilities in these disciplines for patient care, teaching and research in children's hospitals and general hospitals with substantial pediatric facilities.

The value of the Fellowship is \$15,000 annually plus return economy air fare to the location of the study. A larger stipend may be provided for more senior nominees. The stipend is paid quarterly in advance.

The conditions are as follows:

1. The Fellowship may be of one or two years' duration.
2. A fellow must be nominated by the Dean of the Faculty of Medicine and the Chairman of the Department involved or, if no university affiliation, by the Chief of the Service and the Chief Executive Officer of the institution. Names of three medical referees must be provided.
3. A Fellow must be a resident of Canada with the status of a landed immigrant or citizen.
4. A Fellow must have a record of outstanding academic achievements.
5. A Fellow must have a special aptitude for teaching, research and organizational ability.

6. The Fellow must supply an outline of the course of study which he will pursue, the location, the facilities which will be available to him, and who will supervise his study.
7. A written statement from the institution at which the study will be pursued must be provided stating that facilities will be provided and that the Fellow is acceptable.
8. The Fellow must submit a report to the Foundation at the end of the first year (and second year of a two year Fellowship) outlining his activities of the year.
9. Any publications arising from work during the term of the Fellowship must acknowledge the support of the Foundation.
10. The nominating institution must agree that at the completion of the Fellowship, the successful nominee will be appointed to its staff or will retain an existing appointment, and that it is the intent of the institution to continue or establish a department or division or laboratory in the discipline in which the Fellowship is granted. This must be related to the health of children.
11. Members of the staff of the Hospital for Sick Children are eligible for this Fellowship.
12. Any serious deviation from the plan of study requires prior approval from the Foundation.

Application forms may be obtained from: The Hospital for Sick Children Foundation, 555 University Avenue, Toronto, Ontario, M5G 1X8 Canada.

Sponsors of eligible persons are urged to make application for these traineeships which in each instance may be supplemented by other funds available to the institution.

It is to be hoped that the availability of these fellowships will stimulate other foundations, institutions and governmental agencies to provide expanded support for competent, interested young personnel. □

Meeting Announcements

The American Medical Association will sponsor a "Symposium on Fat Emulsions in Parenteral Nutrition," June 5-6, 1975, at the Hyatt Regency O'Hare, Chicago. The symposium meets the criteria for 12 hours of credit, Category 2 for the Physician's Recognition Award of the American Medical Association. Registration, \$30.00 (students free). For further information contact: M. Nagy, AMA Department of Foods and Nutrition, 535 N. Dearborn, Chicago, Illinois 60610.

The Association of Official Analytical Chemists will hold its 89th Annual Meeting October 13-16, 1975, at the Marriott Hotel, Twin Bridges, Washington, D.C. The latest developments in analytical methodology for many commodities and materials important to agricultural and public health areas will be presented and discussed. Nearly 40 firms will exhibit the latest laboratory equipment and supplies.

Registration will continue from 1:00 p.m. Sunday, October 12, through Thurs-

day morning, October 16. The registration fee will be \$5.00 for one day or \$10.00 for two or more days. Anyone interested is invited to attend. For further information, please contact Luther G. Ensminger, Association of Official Analytical Chemists, Box 540, Benjamin Franklin Station, Washington, D.C. 20044.

World famous researchers will meet for a two-day Symposium on Nutritional Disorders of American Women, sponsored by the Institute of Human Nutrition of Columbia University, College of Physicians & Surgeons, at the Commodore Hotel, Lexington Avenue at 42d Street, New York City, November 20 and 21, 1975.

Dr. Myron Winick, Director of the Institute of Human Nutrition, will serve as chairman of the Symposium.

For further information write Director, Institute of Human Nutrition, Columbia University, 511 West 166th Street, New York, N. Y. 10032.

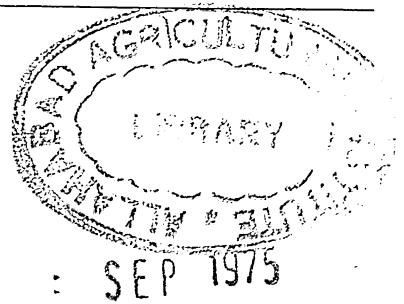
Recent Books

Improvement of Protein Nutrition. Committee on Amino Acids, Food and Nutrition Board, National Research Council. Published by the National Academy of Sciences, Printing and Publishing Office, 2101 Constitution Avenue, Washington, D.C., 20418. Pp. 201. Price \$7.25.

Nutrients in Processed Foods. Proteins. Edited by P. L. White and D. C. Fletcher. Published by Publishing Sciences Group, Inc., Acton, Massachusetts. Pp. 219. Price \$16.00.

Vitamin E

by J. G. Bieri, Ph.D.



Not very long ago vitamin E in human nutrition was described as a "vitamin in search of a disease." Although this statement is no longer accurate, the search in man in contrast to that in animals has turned up a relatively disappointing reward. In order to understand why, it is necessary to have an appreciation of the differences between animals and man in their normal nutritional practices and in the way they are subjected to experimentation. Animals consume a relatively limited number of foodstuffs compared with the array of items in most human diets, thus increasing the chances for animals to develop deficiency. Experimentally, it is possible to place newborn or weaned animals on rations severely deficient in vitamin E, whereas a similar practice could only happen inadvertently in children and would not be prolonged once the error was discovered. As a result of these differences, a myriad of deficiency symptoms has been produced in laboratory and domestic animals, but only a limited number have appeared in man.

Clinical evidence of vitamin E deficiency is almost entirely restricted to premature infants. In several reports, use of infant formulas with an inadequate vitamin E content gave rise to symptoms of irritability

and edema,¹⁻⁴ accompanied by anemia.³ Treatment with α -tocopherol brought about disappearance of all symptoms. An earlier report that vitamin E reversed the megaloblastic anemia seen in protein-calorie malnutrition⁵ has not been confirmed.⁶ Current information indicates the anemia in premature infants is hemolytic resulting in a shortened life span of the red cell.³

It is difficult to produce a vitamin E deficiency in adult man because of the considerable tissue storage of the vitamin and the consequent extended period required for depletion. In the only long-term study, adult men depleted for three years showed no symptoms even though plasma tocopherols fell to very low levels.⁷ A slight decrease in red cell life span was found but there were no manifestations of anemia. Vitamin E deficiency occurs naturally in a high percent of individuals who for various reasons have a defect in their ability to absorb dietary fat.⁸ The largest number are children or young adults with cystic fibrosis. In these patients, although blood levels of the vitamin may be very low, no symptomatology which responds to vitamin E has been observed. Life span of their red cells is often shortened but anemia is not found. A few cases of muscle damage have been noted histologically,⁹ but generally muscle function is not impaired.¹⁰

Dr. Bieri is Chief, Nutritional Biochemistry Section, National Institute of Arthritis, Metabolism and Digestive Diseases, National Institutes of Health, Building 10, Room 5N-102, Bethesda, Maryland 20014.

A limited number of reprints of this article may be obtained from the author. THERE ARE NO REPRINTS OF UNSIGNED REVIEWS.

Vitamin E Status

Evaluation of human vitamin E status is still inaccurate. It has become clear that blood levels of α -tocopherol may not accurately reflect either level of intake or

tissue storage. The extent of red cell hemolysis in vitro is at best a crude estimator of nutritional status; extensive hemolysis indicates a high probability of inadequacy but low hemolysis does not as clearly indicate adequacy of tissue stores.^{11,12} The existence of variables other than vitamin E intake that can affect in vitro hemolysis has added to the uncertainty of this test.¹³⁻¹⁶

Tocopherols in plasma are associated with the lipoproteins and distribute according to the fat content of each fraction.^{17,18} It is well documented that plasma tocopherol levels correlate highly with plasma cholesterol or total lipids.^{17,19} It would also appear that plasma tocopherol values may have little meaning if not accompanied by values for plasma lipids.²⁰ It has been suggested that a ratio of 0.8 mg total tocopherols per gram total plasma lipids would indicate an adequate nutritional status and current information appears to support this.²⁰ Use of this ratio may indicate that some individuals with plasma total tocopherols below 0.5 mg per 100 ml, the commonly accepted satisfactory level, may not be inadequate. At the other end of the spectrum, it will be interesting to evaluate the vitamin E status of individuals with high plasma lipids but with a ratio of less than 0.8 mg tocopherols per gram of lipids, if such a combination exists.

Red cell hemolysis is affected by the cell content of glutathione peroxidase,²¹ shown recently to be a selenium-containing enzyme.²² Newborn infants have reduced red cell glutathione peroxidase activity as well as low plasma α -tocopherol levels,²¹ but it is not known if this is a causal relationship or merely a reflection of physiological immaturity. In adult subjects presumably receiving adequate vitamin E and selenium in their usual diets, erythrocyte glutathione peroxidase activity was remarkably constant with time for each individual, and supplementary α -tocopherol did not change the activity.²¹ In rats and chicks, tissue levels of glutathione peroxidase are related to the dietary selenium

content.²³⁻²⁷ In the presence of adequate selenium, vitamin E status did not affect glutathione peroxidase in liver, kidney, and lung but lower activity was found in adipose and muscle tissue from α -tocopherol deficient animals.²³ Since other nonselenium-containing enzymes were similarly reduced, these results were interpreted as a response of tissues to lipid peroxidation and not to any interaction between selenium and α -tocopherol. It has been suggested that α -tocopherol prevents lipid peroxidation damage to membranes whereas selenium, by being a component of glutathione peroxidase, exerts its protection against peroxidation in the cytosol of cells.²⁵ An interaction between selenium and α -tocopherol has been hypothesized from the observation that dietary α -tocopherol increases the selenide form of selenium in mitochondria and microsomes.²⁸

Absorption and Turnover

The relatively inefficient intestinal absorption of α -tocopherol has been confirmed in several studies. In man, absorption of 20 to 30 percent of small doses was found by lymphatic cannulation,²⁹ while excretion measurements with rats indicated a marked decrease in absorption efficiency as the dose was increased from the microgram to the milligram range.³⁰ Interest in the absorption of other tocopherols, particularly γ -tocopherol, has revealed that contrary to earlier studies which indicated very poor utilization, this vitamin is absorbed to about 85 percent that of α -tocopherol.³¹ Although γ -tocopherol is initially taken up by the tissues as efficiently as is α -tocopherol, it turns over more rapidly and this may account for its low biological activity.^{31,32}

Studies of the rate of accumulation of α -tocopherol in various tissues have shown that after the rapid growth phase of young animals, relatively constant concentrations were maintained in each tissue, generally related to the log of dietary intake.³³ An exception was adipose tissue which progressively accumulated the vitamin. When the vitamin was removed from the diet almost

one-half the α -tocopherol in liver and heart was lost in two weeks, with the remainder disappearing more slowly over a long period. Other tissues, except adipose, also appeared to have two pools of α -tocopherol. It has been suggested that the relatively nonlabile fraction may represent α -tocopherol in cellular membranes.³⁴

Enzyme Function

At the molecular level, search for an unequivocal, direct involvement of α -tocopherol in enzyme functioning has continued unabated. Most attention recently centered on a possible role for vitamin E in heme synthesis, with evidence that in deficiency the activity of two enzymes may be altered.³⁵ In bone marrow, δ -aminolevulinic acid (ALA) synthetase was decreased and in liver ALA dehydratase was reduced. Other investigators exploring this possible involvement of α -tocopherol in heme synthesis have been unable to relate the deficient state with reduced heme or cytochrome synthesis.^{36,37} It would appear that variables other than vitamin E status may have caused these discrepant findings; thus conclusive evidence for a regulatory role of the vitamin in these systems is lacking. Another liver system, the microsomal enzyme drug hydroxylating complex, has been shown to be affected by the vitamin E status of the animal.³⁸ Reduced activity in deficient rats is restored to normal 12 hours after an oral dose of the vitamin. Regulation of this system is complex, being affected by numerous inducers and by steroid hormones, so that the mechanism of the vitamin E involvement at present is obscure.³⁸ Oxidative demethylation, another microsomal drug metabolizing system, was not affected by vitamin E status but a slight decrease in drug induction was found.³⁹

Liver xanthine oxidase was shown to be increased in vitamin E-deficient rabbits in 1953.⁴⁰ Subsequent studies found that this is not due to loss of an inhibitor or to an activator, but is caused by accumulation of the enzyme due to accelerated synthesis.⁴¹ In contrast to the increased synthesis of

this enzyme in rabbits, a marked reduction in overall protein synthesis was found in liver microsomes and polysomes from vitamin E-deficient rats.⁴² In the latter study, however, abnormal membrane lipid peroxidation was induced by feeding a high level of polyunsaturated fatty acids. One mechanism by which lipid peroxidation may occur in membranes was shown by the generation of free radicals during the oxidation of TPNH by liver microsomes.⁴³ In this model system using the erythrocyte membrane, free radical damage could be prevented by prior feeding of vitamin E to the donor animal.

Increased amounts of arachidonic acid have been found in several tissues of vitamin E-deficient rats.⁴⁴ Examination of liver for a possible explanation revealed that microsomes from deficient animals synthesized more arachidonic acid from γ -linolenic acid and also more γ -linolenic from linoleic acid.⁴⁵ A variety of enzymes related to the respiratory chain were found to be unaffected by vitamin E status, confirming earlier studies which showed that vitamin E is not directly involved in the electron transport mechanism.⁴⁶ Thorough study of the accelerated turnover of creatine kinase in vitamin E-deficient rabbit muscle showed no difference in intrinsic protein synthetic activity from normal muscle, but deficient muscle had increased numbers of polysomes.⁴⁷ It remains to be demonstrated that any enzyme reaction has a specific requirement for α -tocopherol although many of the observed enhancements or inhibitions due to vitamin E status have not been adequately explained.

Toxicity Protection

Evidence from animal studies over many years showed that dietary vitamin E affords protection from various chemical toxicants.⁴⁸ Recent interest centered on preventing toxic effects of atmospheric pollutants. Just as it was shown in 1958 that supplementary α -tocopherol would decrease toxicity from a high oxygen atmosphere,⁴⁹ similar protection from

ozone and nitrous oxide has also been demonstrated.⁵⁰⁻⁵² Such oxidants can cause lipid peroxidation damage in rats, but the relevance of these results to man is not clear at this time. It is known that other protective mechanisms exist in the lung, e.g. glucose-6-phosphate dehydrogenase, glutathione peroxidase, and superoxide dismutase,^{53,54} but the relative effectiveness of α -tocopherol and these enzyme systems is not clear.

Large Doses

Health claims for large dietary supplements of vitamin E still remain unsubstantiated, and a recent review of the subject has led the Food and Nutrition Board to state that such claims are not adequately documented.⁵⁵ In chicks, supplements of α -tocopherol above the normal dietary level produced an enhanced immune response, while α -tocopherol in vitro had a similar effect on normal mouse spleen cells.^{56,57} In view of a similar effect of dietary vitamin A on the immune response in mice,⁵⁸ the possible action of vitamin E in the immune system remains obscure.

Considerable numbers of individuals continue to self-dose themselves regularly with relatively large doses of vitamin E for various reasons. Possible toxicity of such supplements has led to experiments in animals, with mixed results. In the chick, depressed growth, interference in thyroid function, and increased requirements for vitamin D and vitamin K were found.⁵⁹ In rats, liver total lipid and cholesterol were increased by high vitamin E dosage, and tissue fatty acid patterns were altered.⁶⁰ In man, minor complaints of nausea and intestinal distress appeared in some subjects ingesting more than 300 IU per day,⁶¹ and a variety of other nonspecific complaints have also been noted.⁶² Isolated reports of other symptoms can be found in the medical literature but the general impression is that for most individuals daily doses below 300 IU are innocuous.

Recommended Allowance

A recommended dietary allowance for vitamin E was first set in 1965.⁶³ In retro-

spect, this initial effort set values that were unrealistically high and most U.S. diets adequate in all other nutrients would not meet the allowance. In the light of reports subsequent to 1965, the 1974 edition of Recommended Dietary Allowances⁶⁴ reduced the allowances considerably. It was pointed out that a fixed allowance for any age and sex group is not realistic, but that a range of 10 to 20 IU of total vitamin E activity should be present in adult diets supplying 1800 to 3000 kcal. The tabulated values should be recognized as averages which may or may not be adequate depending on other components of the diet, primarily the polyunsaturated fatty acid (PUFA) content. Generally, however, as the PUFA in diets increase, so does the vitamin E content.

Because the recommended dietary allowance for vitamin E is primarily determined by the amount of the vitamin in balanced diets consumed by healthy individuals, the accuracy of determining vitamin E in foodstuffs or diets is of the utmost importance. Analyses of diets using newer analytical procedures found that the vitamin E content of U.S. and Canadian diets is not as high as proposed in 1963.⁶⁵ Three studies showed that typical balanced diets of about 2500 kcal average from 6.4 to 9.0 mg (9.5 to 13.4 IU) of α -tocopherol daily.⁶⁶⁻⁶⁸ In addition, U.S. diets now contain considerably more γ -tocopherol than α -tocopherol,⁶⁷ due to the trend over the past 30 years to substitute vegetable fats and oils for animal fats.⁶⁹ The predominant vegetable oil, soybean oil, contains about six times more γ - than α -tocopherol. Even though γ -tocopherol has only about 10 percent of the biological activity of α -tocopherol,⁷⁰ the large amount of γ -tocopherol in diets was estimated to contribute as much as 20 percent of the total vitamin E activity.⁶⁷ Furthermore, more detailed analyses of whole cereal grains found that barley, oats, and rye contain from 0.9 to 1.7 mg per 100 g of other vitamers, α -tocotrienol and β -tocopherol,⁷¹ which are about 40 percent as active as α -tocopherol. The 1974 Recommended Dietary Allowances state that in calculating

the vitamin E content of mixed diets, values for α -tocopherol should be multiplied by a factor of 1.2 to give a more accurate estimation of their total vitamin E activity, expressed as α -tocopherol equivalent.^{6,4}

Early animal studies clearly showed that the vitamin E requirement increased with increased intake of polyunsaturated fatty acids (PUFA). In an initial effort to relate these two dietary variables it was suggested that a fixed ratio, termed the E:PUFA ratio, might be established to define dietary vitamin E adequacy.^{6,5} Unfortunately, the initially suggested ratio was accepted by many nutritionists before it was adequately tested experimentally. Subsequently it has been found with several species that a fixed E:PUFA ratio does not apply in a variety of experimental conditions, and that factors in the diet other than the PUFA content affect the vitamin E status.^{7,2} For example, rats ingesting a diet containing 20 percent of safflower oil (E:PUFA=0.34) had about the same tissue α -tocopherol content as rats ingesting a diet with 20 percent of a modified corn oil with a ratio of 0.25.^{7,3} It would appear that until more information is available on dietary factors that influence vitamin E nutritional status, a simple ratio should not be used.^{6,8,7,2}

Novel Effects

Whereas α -tocopherol appears to function primarily as a stabilizer of lipid structures in animal tissues, observations of some novel effects of the vitamin on the fresh water rotifer, *Asplanchna sieboldi* indicate a more complex, metabolic action.^{7,4} Addition of α -tocopherol to the medium produced a significantly larger adult body size, and also caused the normally parthenogenic females to have an altered phenotype and produce male offspring. These effects were very specific for α -tocopherol, with other tocopherols or antioxidants being inactive. Earlier, a growth-stimulating effect of vitamin E on the insect larvae, *Agria affinis*, was reported.^{7,5} The observation that α -tocopherol quinone was just as effective as α -tocopherol in increasing longevity in

nematodes^{7,6} suggests that some of these effects on lower animals may be due to structural aspects of these compounds unrelated to the functional hydroxyl group of α -tocopherol.

Structural Analogs

Structure-function relationships received renewed interest with the synthesis of various amino and substituted amino analogs.^{7,7} When the hydroxyl group of the tocopherols was replaced by an amino group, the resulting tocopheramines had the same biological activity as the corresponding tocopherols. When the hydroxyl group was replaced by a methyl-amino group ($\text{CH}_3\text{-NH-}$), then these analogs of β - and γ -tocopherols became fully as active as α -tocopherol, and were stored in tissues as well as or better than α -tocopherol.^{7,8} These studies indicate that three methyl groups around the chroman ring are essential for full activity but that the position of the methyls is not critical. Earlier studies had shown that either a shorter or unsaturated side chain reduced activity. \square

1. H. Hassan, S. A. Hashim, T. B. Van Itallie, and W. H. Sebrell, *Am. J. Clin. Nutrition* 19: 147-157, 1966
2. F. A. Oski and L. A. Barness, *J. Pediat.* 70: 211-220, 1967
3. J. H. Ritchie, M. B. Fish, V. McMasters, and M. Grossman, *New Engl. J. Med.* 279: 1185-1190, 1968
4. M. A. Chadd and A. J. Fraser, *Int. Z. Vitaminforsch.* 40: 604-609, 1970
5. A. S. Majaj, J. S. Dinning, S. A. Azzam, and W. J. Darby, *Am. J. Clin. Nutrition* 12: 374-379, 1963
6. S. J. Baker, S. M. Pereira, and A. Begum, *Blood* 32: 717-725, 1968
7. M. K. Horwitt, *Am. J. Clin. Nutrition* 8: 451-461, 1960
8. H. J. Binder and H. M. Spiro, *Am. J. Clin. Nutrition* 20: 594-601, 1967
9. T. Weinberg, H. H. Gordon, E. H. Oppenheimer, and H. M. Nitowsky, *Am. J. Path.* 34: 565-566, 1958
10. S. Levin, M. H. Gordon, H. M. Nitowsky, C. Goldman, P. di Sant'Agnese, and H. H. Gordon, *Pediatrics* 27: 578-588, 1961
11. J. G. Bieri and R. K. H. Poukka, *Int. Z. Vitaminforsch.* 40: 344-350, 1970

12. J. T. Dodge, G. Cohen, H. J. Kayden, and G. B. Phillips, *J. Clin. Invest.* 46: 357-358, 1967
13. D. K. Melhorn, S. Gross, G. A. Lake, and J. A. Leu, *Blood* 37: 438-446, 1971
14. J. Stocks and T. L. Dormandy, *Brit. J. Haematol.* 20: 95-111, 1971
15. J. Stocks, M. Kemp, and T. L. Dormandy, *Lancet* i: 266-269, 1971
16. L. G. Macdougall, *J. Pediat.* 80: 775-782, 1972
17. T. Davies, J. Kelleher, and M. S. Losowsky, *Clin. Chim. Acta* 24: 431-436, 1969
18. I. R. Peake, H. G. Windmueller, and J. G. Bieri, *Biochim. Biophys. Acta* 260: 679-688, 1972
19. H. M. Rubenstein, A. A. Dietz, and R. Srinivasan, *Clin. Chim. Acta* 23: 1-6, 1969
20. M. K. Horwitt, C. C. Harvey, C. H. Dahm, Jr., and M. T. Searcy, *Ann. N. Y. Acad. Sci.* 203: 223-236, 1972
21. P. M. Emerson, D. Y. Mason, and J. E. Cuthbert, *Brit. J. Haematol.* 22: 667-680, 1972
22. J. T. Rotruck, A. L. Pope, H. E. Ganther, A. B. Swanson, D. G. Hafeman, and W. G. Hoekstra, *Science* 179: 588-590, 1973
23. C. K. Chow, K. Reddy, and A. L. Tappel, *J. Nutrition* 103: 618-624, 1973
24. C. K. Chow and A. L. Tappel, *J. Nutrition* 104: 444-451, 1974
25. T. Noguchi, A. H. Canter, and M. L. Scott, *J. Nutrition* 103: 1502-1511, 1973
26. D. G. Hafeman, R. A. Sunde, and W. G. Hoekstra, *J. Nutrition* 104: 580-587, 1974
27. S. T. Omaye and A. L. Tappel, *J. Nutrition* 104: 747-753, 1974
28. A. T. Diplock, H. Baum, and J. A. Lucy, *Biochem. J.* 123: 721-729, 1971
29. R. Blomstrand and L. Forsgren, *Int. Z. Vitaminforsch.* 38: 328-344, 1968
30. M. S. Losowsky, J. Kelleher, B. E. Walker, T. Davies, and C. L. Smith, *Ann. N. Y. Acad. Sci.* 203: 212-222, 1972
31. U. Gloor, J. Würsch, U. Schwieter, and O. Wiss, *Helv. Chim. Acta* 49: 2303-2312, 1966
32. I. R. Peake and J. G. Bieri, *J. Nutrition* 101: 1615-1622, 1971
33. J. G. Bieri, *Ann. N. Y. Acad. Sci.* 203: 181-191, 1972
34. I. Molenaar, C. E. Hulstaert, and J. Vos, *Proc. Nutrition Soc.* 32: 249-254, 1973
35. P. I. Caasi, J. W. Hauswirth, and P. P. Nair, *Ann. N. Y. Acad. Sci.* 203: 93-102, 1972
36. M. P. Carpenter, *Ann. N. Y. Acad. Sci.* 203: 81-92, 1972
37. A. T. Diplock, *Am. J. Clin. Nutrition* 27: 995-1004, 1974
38. M. P. Carpenter and C. N. Howard, Jr., *Am. J. Clin. Nutrition* 27: 966-979, 1974
39. K. Bernhard, R. Markstein, and W. Zimmerli, *Helv. Chim. Acta* 54: 2568-2572, 1971
40. J. S. Dinning, *J. Biol. Chem.* 202: 213-215, 1953
41. G. L. Catignani, F. Chytil, and W. J. Darby, *Proc. Nat. Acad. Sci. USA* 71: 1966-1968, 1974
42. U. Reiss and A. L. Tappel, *Biochim. Biophys. Acta* 312: 608-615, 1973
43. P. M. Pfeifer and P. B. McCay, *J. Biol. Chem.* 246: 6401-6408, 1971
44. L. A. Witting and M. K. Horwitt, *Lipids* 2: 89-96, 1967
45. K. Bernhard and R. Markstein, *Helv. Chim. Acta* 55: 519-526, 1972
46. J. Green in *The Vitamins*. W. H. Sebrell, Jr. and R. S. Harris, Editors, vol. 5, pp. 259-272. Academic Press, New York, 1972
47. R. E. Olson, *Am. J. Clin. Nutrition* 27: 1117-1129, 1974
48. E. L. Hove, *Am. J. Clin. Nutrition* 3: 328-336, 1955
49. D. W. Taylor, *J. Physiol.* 140: 37-47, 1958
50. H. V. Thomas, P. K. Mueller, and R. L. Lyman, *Science* 159: 532-534, 1968
51. B. D. Goldstein, R. D. Buchley, R. Cardenas, and O. J. Balchum, *Science* 169: 605-606, 1970
52. J. N. Roehm, J. G. Hadley, and D. B. Menzel, *Arch. Int. Med.* 128: 88-93, 1971
53. C. K. Chow and A. L. Tappel, *Lipids* 7: 518-524, 1972
54. D. F. Tierney, *Fed. Proc.* 33: 2232-2237, 1974
55. Supplementation of human diets with vitamin E. National Academy of Sciences, Washington, D. C., 1973
56. R. P. Tengerdy and C. F. Nockles, *Poultry Sci.* 52: 778-783, 1973
57. P. A. Campbell, H. R. Cooper, R. H. Heinzerling, and R. P. Tengerdy, *Proc. Soc. Exp. Biol. Med.* 146: 465-469, 1974
58. B. E. Cohen and I. K. Cohen, *Surg. Forum* 24: 276-278, 1973
59. B. E. March, E. Wong, L. Seier, J. Sim, and J. Biely, *J. Nutrition* 103: 371-377, 1973
60. R. B. Alfin-Slater, L. Aftergood, and S. Kishineff, Abstract IX International Congress of Nutrition, 191, 1972
61. A. B. Vogelsang, E. V. Shute, and W. E. Shute, *Med. Record* 160: 279-284, 1947

62. R. A. King, *J. Bone Joint Surg.* 31B: 443, 1949
63. *Recommended Dietary Allowances*. Seventh edition. National Academy of Sciences, Washington, D. C., 1965
64. *Recommended Dietary Allowances*. Eighth edition. National Academy of Sciences, Washington, D. C., 1974
65. P. L. Harris and N. D. Embree, *Am. J. Clin. Nutrition* 13: 385-392, 1963
66. R. H. Bunnell, J. Keating, A. Quaresimo, and G. K. Parman, *Am. J. Clin. Nutrition* 17: 1-10, 1965
67. J. G. Bieri and R. Poukka Evarts, *J. Am. Dietet. Assn.* 62: 147-151, 1973
68. J. N. Thompson, J. L. Beare-Rogers, P. Erdody, and D. C. Smith, *Am. J. Clin. Nutrition* 26: 1349-1354, 1973
69. J. G. Bieri and R. Poukka Evarts, *Am. J. Clin. Nutrition* 27: 980-986, 1974
70. J. G. Bieri and R. Poukka Evarts, *J. Nutrition* 104: 850-857, 1974
71. H. T. Slover, *Lipids* 6: 291-296, 1971
72. F. C. Jager, *Ann. N. Y. Acad. Sci.* 203: 199-211, 1972
73. J. G. Bieri and R. Poukka Evarts, *J. Am. Dietet. Assn.* (in press)
74. J. J. Gilbert, *Am. J. Clin. Nutrition* 27: 1005-1016, 1974
75. H. L. House, *J. Insect Physiol.* 12: 409-417, 1966
76. J. Epstein and D. Gershon, *Mech. Age. Devel.* 1: 257-264, 1972
77. U. Schwieter, R. Tamm, H. Weiser, and O. Wiss, *Helv. Chim. Acta* 49: 2297-2303, 1966
78. J. G. Bieri in *The Fat-Soluble Vitamins*. H. F. DeLuca and J. W. Suttie, Editors, pp. 307-316. The University of Wisconsin Press, Madison, Wisconsin, 1969

Nutrition Reviews

authoritative articles by distinguished international scientists • competent, critical, and knowledgeable reviews • interpretative summaries of important original clinical and experimental research • selected excerpts from classic publications marking milestones in nutrition research • informative special reports on government and organization actions • contributing editors review journals of widespread interest covering biochemistry, food science, public health, toxicology, dentistry, pediatrics, internal medicine, gastroenterology and other clinical specialties •

Send subscription and renewal requests to:

THE NUTRITION FOUNDATION
489 Fifth Avenue
New York, New York 10017

\$12.00 per year

\$6.00 for students

BLUNTED RESPONSE TO ORAL ALANINE FEEDING IN SMALL-FOR-GESTATIONAL-AGE INFANTS

Small-for-gestational-age infants show a defect in gluconeogenesis during the first day of life.

Key Words: glucagon, glucose, small birth weight, prematurity, placental failure, alanine, gluconeogenesis, infant nutrition

Small-for-gestational-age infants (below the tenth percentile based upon menstrual history and the method of Dubowitz et al.)¹ usually represent intrauterine growth retardation due to placental insufficiency which is also associated with toxemia during pregnancy. Twenty-one such infants were compared to 26 infants who were between the tenth and 90th percentile in weight.² All infants were studied within the first 96 hours of birth; half of the infants were studied before any feeding, and the remaining infants were examined after several feedings. All were given a test meal of 500 mg per kilogram of L-alanine orally. Blood samples were obtained before and after 30 and 60 minutes under acceptable conditions to permit analysis for glucose, alanine, glucagon, and insulin.

Alanine feeding was studied because this amino acid stimulates glucagon secretion and enhances gluconeogenesis in adults and in full term newborn infants. Small-for-gestational-age infants have a higher incidence of hypoglycemia, which is attributed to both decreased hepatic glycogen reserve and impaired hepatic gluconeogenesis.³

In studies conducted during the first 24 hours of life, baseline plasma alanine levels were significantly higher in the small-for-age infants ($1030 \pm 60 \mu\text{M}$) than in the normal infants ($590 \pm 90 \mu\text{M}$). After alanine feeding, both groups showed similar increases in plasma alanine levels, with the small-for-age group maintaining their relatively higher levels. Baseline glucagon levels were slightly, but not significantly, higher

in the small-for-age group of infants; after alanine feeding there was an increase in plasma glucagon levels in both groups. In the case of insulin, the baseline values were similar in the two groups of infants, and rose significantly after alanine feeding only in the normal infants.

Blood glucose values were similar in the two groups of infants; however, only the normal size group responded with an increase of blood sugar to alanine administration, the small-for-age group showing if anything a decrease of blood sugar.

In contrast to the above results obtained during the first 24 hours of life, in older infants (25 to 96 hours), baseline values for plasma alanine, glucose, and alanine were the same in the two groups of infants. There was no change in the glucose, glucagon, or insulin levels after alanine feeding in either group. Baseline glucose levels were higher, however, in the older than in the younger infants.

The data show that in very early life (less than 24 hours) the small-for-gestation-age infants do not enhance glucose output after ingestion of a gluconeogenic amino acid, in contrast to normal infants of the same age. In an editorial comment on the article of Williams et al.², Hahn⁴ points out that upon delivery the small-for-gestational-age infant lacks supplies of glycogen and fat, having been malnourished in utero for some time. Nevertheless, even in this group there is a glucagon response to alanine feeding in the sequence of events believed to occur immediately after birth, namely: a decrease in blood glucose; an increase in glucagon release; activation of liver and fat adenylcyclase; activation of glycogenolysis

and lipolysis; and induction of gluconeogenic enzymes.

Hahn also refers to the work of Reisner, et al.⁵ who found a rise in blood sugar in response to glucagon, even in the small-for-gestational age infants, but points out some differences in the protocols from those of Williams et al. Hahn emphasizes the importance of the glucagon response toward setting in motion the adjustment of metabolism in the newborn infant, and suggests that glucose infusion for hypoglycemia in the newborn may be detrimental to metabolic adjustment since it suppresses glucagon release. □

1. L. M. S. Dubowitz, V. Dubowitz, and C. Goldbert: Clinical Assessment of Gestational Age

in the Newborn Infant. *J. Pediat.* 77:1-10, 1970

2. P. R. Williams, R. H. Fiser, Jr., M. A. Sperling, and W. Oh: Effects of Oral Alanine Feeding on Blood Glucose, Plasma Glucagon and Insulin Concentrations in Small-For-Gestation-Age Infants. *New Engl. J. Med.* 292: 612-614, 1975
3. M. Cornblath, S. H. Wybregt, G. S. Baens, and R. I. Klein: Symptomatic Neonatal Hypoglycemia. Studies of Carbohydrate Metabolism in the Newborn Infant. VIII. *Pediatrics* 33: 388-402, 1964
4. P. Hahn: Nurture of the Newborn. *New Engl. J. Med.* 292: 642-643, 1975
5. S. H. Reisner, J. V. Aranda, E. Colle, A. Papa-georgiou, D. Schiff, C. R. Scriver, and L. Stern: The Effect of Intravenous Glucagon on Plasma Amino Acids in the Newborn. *Pediat. Res.* 7: 184-191, 1973

GASTRIC EMPTYING, PANCREATIC AND BILIARY SECRETION DURING DIGESTION

The type of meal determines the duration of gastric emptying which in turn influences the duration of pancreatic and bile secretions in normal human beings. It may be that the meal flow is the stimulus which evokes the cholecystokinin-pancreozymin release from the small intestine, which in turn, determines the quantities of secretion of the pancreas and biliary system.

Key Words: trypsin, lipase, bile acid, gastric emptying

One of the most difficult nutritional problems to treat in medicine is the short-bowel syndrome, not only because of the failure of the gut to absorb materials properly, but also because of body fluid and nutrient loss into the gut lumen. The problems are directly related to the length of small intestine which is functional and the ability of this length to adapt its secretory and absorptive functions. Those individuals with small lengths which fail to adapt properly gradually lose body weight. Fluid loss and failure to absorb are related to changes in transport function of the gut and are compounded by hypersecretion of the stomach. Sometimes selective gastric resection improves clinical conditions. Hopefully, a physiologic approach to therapy can be

defined when more is known about arrangements but little quantitative knowledge is available even in normal people. A recent paper is a step forward in this regard. Brunner and his co-workers describe gastrointestinal reactions to such stimuli as caloric content and volume of foods, and their relationships to gastric emptying, pancreatic, and bile secretion.¹ Although the study was not done to form the basis for treatment of the short-bowel syndrome, perhaps the data can eventually be applied to formulate a logical dietary approach in the treatment of this disorder.

The investigators set out to quantify and correlate digestive mechanisms under physiologic circumstances. They measured simultaneously, gastric emptying and total outputs of biliary constituents and pancreatic enzymes in response to meals.

Healthy subjects consumed three liquid test meals and studies were done for 12 to 24 hour periods. Eighteen volunteers were assigned to three groups of six subjects each. Each group ingested a liquid test meal of different caloric content. The nature of the experiment was such that the meals had to be given via a gastric tube in three equal caloric portions at 8, 12, and 6 PM. The low calorie (LC) was 20; the medium calorie (MC) was 30; and the high calorie (HC) was 40 kcal per kilogram of body weight. The meals were 40 percent fat, 46 percent carbohydrate (dextrose), and 13 percent protein which was skim milk powder. To quantitate pancreatic and bile secretion, isotonic saline containing polyethylene glycol as a volume indicator and radioactive cholesterol was perfused in the second portion of the duodenum at a rate of 2 ml per minute. Sampling of intestinal contents was achieved through ports in the tube near the ligament of Treitz and 30 cm distally in the jejunum. Radioactive chromium was placed in the test meals so that the volume of the meal passing the duodenum could be assessed. Bile acid kinetics and pool size were assessed by measuring the appearance of radioactive bile salts in the intestinal lumen. The radioactive bile salts had been given four days prior to the perfusion. During the study there was no significant reflux of duodenal contents into the stomach.

Gastric emptying was exponential during most of the digestive period, followed by a terminal phase of rapid emptying. The time for emptying was greater for meals higher in calories and volume. In only two of the 18 subjects did the stomach empty completely between successive meals.

Bile acid output increased rapidly above the basal secretion immediately after the first meal. It continued to remain elevated throughout the rest of the study period of 12 hours and then gradually declined after the 6 PM meal.

The hourly trypsin output during the first 18 hours after ingestion of test meals was similar in all groups. There was a significant increase above the basal secretion

after the first meal. This also tended to remain elevated for more than 12 hours. The trypsin output in the low calorie group tended to return to baseline sooner than it did in the medium and high calorie groups. During the first 12 hours in which the three meals were ingested, total bile acid and cholesterol outputs were significantly higher than outputs in the second 12 hours in all groups. The caloric and volume size of the meals apparently did not affect the total bile acid and cholesterol output, which were similar for all three groups of subjects. In the second 12 hours, however, bile acid and cholesterol outputs were significantly lower in the LC group than in the MC and HC groups. This resulted in a lower 24-hour output of bile acid and cholesterol in the LC group. The bile acid pool size was similar in all groups and correlated very poorly with body weight ($r=0.53$) or body surface ($r=0.55$). Bile acid pool circulated approximately four times during the 24 hours of observation in the LC group and about six times in the MC and HC groups.

Trypsin and lipase, as representatives of pancreatic enzyme output, were significantly higher during the first 12 hours than in the subsequent 12 hours in the LC group. In the subsequent 12 hours in which no meals were fed, the output of these enzymes decreased in the LC group but remained elevated in the MC and HC groups. Thus, less of these enzymes were secreted in the LC group.

These data show that the nature of the meal controlled the duration of gastric emptying. This in turn, determined the amounts and duration of pancreatic and biliary secretion (the arrival of food into the proximal small intestine releases gastrointestinal hormones that stimulate gallbladder contraction, bile secretion, and pancreatic enzyme secretion).

Do these data suggest approaches to treatment of the short-bowel syndrome? Since the total output of bile and pancreatic secretion depends largely on the number of hours required for gastric emptying, it may well be that small meals

of lower caloric density might be most beneficial in the treatment of the small-bowel syndrome rather than high volume, high calorie feedings which in the normal individual tend to maintain a persistent elevation of pancreatic and bile secretion. Similar studies, however, are needed in individuals with fluid loss problems through the gastrointestinal tract. Although loss of the small intestine most likely means a loss of hormones or other substances which turn off gastric, bile, and pancreatic secre-

tion, this should not alter the search for a physiologic approach for dietary therapy. With more data, perhaps the loss of fluid and nutrients can be successfully overcome.

1. H. Brunner, T. C. Northfield, A. F. Hofmann, V. L. W. Go, and W. H. J. Summerskill: Gastric Emptying and Secretion of Bile Acids, Cholesterol, and Pancreatic Enzymes During Digestion. Duodenal Perfusion Studies in Healthy Subjects. *Mayo Clin. Proc.* 49: 851-860, 1974

ENDEMIC GOITER AND ANTITHYROID AGENTS

Although endemic goiter undoubtedly responds to iodination programs there is now considerable evidence that antithyroid factors play an important role.

Key Words: goiter, iodine, *Escherichia coli*, antithyroid agents

There is abundant evidence that the iodination of salt markedly reduces the prevalence of endemic goiter. Thus, endemic goiter is generally considered to be the result of iodine deficiency. Accumulating evidence, however, emphasizes the role of antithyroid agents which apparently play an important part either by modifying the iodine or by preventing iodination from being completely effective.

Working in the sub-Himalayan region at the turn of the present century, Sir Robert McCarrison attributed the goiter there to a living agent. The heroic experiment wherein he along with other volunteers drank the suspended matter from the local streams, believed by him to be polluted, and subsequently developed a goiter, will forever remain a classic.^{1,2} Although Greenwald tried to keep up the banner of this theme,³ not much attention has been paid to this aspect. Even where this etiological factor is referred to,⁴ one can discern an underlying note of skepticism.

Extensive studies in Cali, Colombia, by Gaitan⁵ apparently demonstrated that the occurrence of goiter could not be correlated with differences in iodine consumption but could be correlated with the

source of drinking water. Water from districts with a high incidence of goiter was found to have "thiourea-like antithyroid activity". In experimental animals the water caused enlarged thyroids, impaired ¹³¹I uptake, decreased thyroidal iodine content, and impaired the formation of diiodotyrosine. Dichloromethane extracts of the goitrogenic waters revealed a group of saturated and unsaturated aliphatic hydrocarbons, some of which were sulfurated, which were fractionated and tested for biological activity. Three sulfurated hydrocarbons with marked antithyroid activity were obtained.

Gaitan and co-workers suspect that such water-borne goitrogens are causally related to the endemic goiter in the Cauca Valley of Colombia. If the goitrogens are of geologic origin, they are presumably from marine sedentary rocks. These rock formations, rich in hydrocarbons, are sporadically exposed on both sides of the Cauca Valley.

Endemic goiter has been identified in areas of Kentucky and West Virginia. Vought and his colleagues^{6,7} found no evidence of dietary deficiency of iodine in these areas. The supply of drinking water was not protected in these areas nor were there proper facilities for sewage disposal.

Vought et al.⁸ studied the effects of extracts of *Escherichia coli* on rats in an attempt to evaluate the possibility that bacterial infection might play a role in goitrogenesis.

E. coli Poly A, Poly B, Poly C, and *E. coli* isolated from a polluted spring in Richmond county, Virginia, were used for the study. The polluted spring supplied a small population claimed to be highly goitrous. Bacterial cultures were prepared by either vat fermentation described in detail in the report or by conventional incubation procedures. The cultures were centrifuged, the supernate was filtered, and the filtrate lyophilized. The crude extract was fractionated by ultrafiltration and fractions with molecular weight between 5×10^4 and 1×10^5 were routinely used for the testing of antithyroid activity.

Young male rats were placed on a low iodide diet for ten to 14 days. The test material was then injected subcutaneously. The control animals received distilled water or the residue from sterile broth, in place of the test material. Sodium ^{131}I was injected intraperitoneally half an hour later. The thyroid was counted after three hours. The neck probe contained a preamplifier which was fed to a rate meter. This helped in achieving a better reproducibility.

Antithyroid activity reflected as a reduced uptake of ^{131}I by the thyroid was present in the 5×10^4 to 10×10^4 molecular weight fraction of the cell-free extract of the *E. coli*. The authors are cautious in their claims but the results do indicate that *E. coli* may produce goitrogenic agents. As Stanbury⁹ stated, such goitrogens are probably low in concentration and may normally be ineffective but may achieve significance when the supply of iodide is limited.

Many of the world's goitrous zones today are in the developing nations where bacterial pollution of drinking water and food is common. Studies on the effects of

bacteria as well as minor constituents of the water supply may provide the explanation for the prevalence of goiter which does not seem possible on the basis of iodine deficiency alone.¹⁰⁻¹² These kinds of studies may also be of significance in deciding upon appropriate levels of iodide fortification. □

1. R. McCarrison: Observations on Endemic Cretinism in the Chitral and Gilgit Valleys. *Lancet* II: 1570-1577, 1908
2. R. McCarrison: Adventures in Research. *Trans. Med. Soc. London* 60: 46-71, 1937
3. I. Greenwald: The Significance of the History of Goiter for the Etiology of the Disease. *Am. J. Clin. Nutrition* 8: 801-807, 1960
4. Endemic Goiter. World Health Organization Technical Report Series No. 44, 1960
5. E. Gaitan: Water-Borne Goitrogens and Their Role in the Etiology of Endemic Goiter. *World Rev. Nutrition Dietet.* 17: 53-90, 1973
6. W. T. London, D. A. Koutras, A. Pressman, and R. L. Vought: Epidemiologic and Metabolic Studies of a Goiter Endemic in Eastern Kentucky. *J. Clin. Endocrinol. Metab.* 25: 1091-1100, 1965
7. R. L. Vought, W. T. London, and G. E. T. Stebbing: Endemic Goiter in Northern Virginia. *J. Clin. Endocrinol. Metab.* 27: 1381-1389, 1967
8. R. L. Vought, F. A. Brown, and K. H. Sibinovic: Antithyroid Compound(s) Produced by *Escherichia coli*: Preliminary Report. *J. Clin. Endocrinol. Metab.* 38: 861-865, 1974
9. J. B. Stanbury in *Clinical Endocrinology*. E. B. Astwood and C. E. Cassidy, Editors, pp. 195-209. Grune and Stratton, New York, 1968
10. Goiter Among Ceylonese and Nigerians. *Nutrition Reviews* 26: 77-80, 1968
11. Dietary Iodine and Goiter in Ceylon. *Nutrition Reviews* 27: 108-110, 1969
12. B. Malamos, D. A. Koutras, G. A. Rigopoulos, P. D. Papápetrou, E. Gougas, H. Kelperi, C. Moraitopoulos, E. Davi, and J. Leonaropoulos: Endemic Goiter in Greece: Some New Epidemiologic Studies. *J. Clin. Endocrinol. Metab.* 32: 130-139, 1971

TYPE III HYPERLIPOPROTEINEMIA

Type III hyperlipoproteinemia is a disease of adults with an increase in blood cholesterol, triglycerides, and very low density lipoproteins. Xanthomas are present with the classic xanthoma striata palmaris most evident. Subjects have, most commonly, ischemic heart disease, peripheral vascular disease, or a combination of both. It is one of the most responsive disorders to dietary management.

Key Words: plasma cholesterol, triglycerides, lipoproteins, xanthomas, hyperlipoproteinemia

Type III hyperlipoproteinemia is diagnosed by its lipoprotein pattern, elevated plasma cholesterol, and triglyceride concentrations. Cutaneous and subcutaneous xanthomas are present and a high hazard for premature atherosclerosis has been shown. Type III disease is not rare. Since it responds well to dietary therapy and since it can be most easily diagnosed from its clinical features, its recognition is important. A report of 50 patients with Type III disease has been summarized from clinical and biochemical aspects.¹ Subjects were over 19 years of age and initial levels of blood triglycerides exceeded 190 mg per 100 ml. The diagnosis was established from biochemical data when the ratio of the difference between the concentration of cholesterol in whole plasma and the ultracentrifugally isolated plasma fractions of density greater than 1.006 was divided by the plasma triglyceride concentration and the ratio found to be not less than 0.3. The chemical diagnosis was suspect if trigly-

cerides were less than 150 or greater than 1000 mg per 100 ml. Furthermore, when the ratio was between 0.25 and 0.29 it was felt that "possible type III hyperlipoproteinemia" was the diagnosis. The age of onset of Type III was arbitrarily defined as the age at which the patient was first found to have hyperlipidemia, xanthomas, or definite vascular disease.

Subjects with definite Type III disease were between the ages of 23 to 70 at the time of diagnosis; the mean age in men was 40 and in women it was 50. The lipid and lipoprotein concentrations in the untreated state are shown in Table 1. The most obvious abnormality in lipoprotein concentrations was the marked elevation in the very low density lipoproteins; control values were only 14 to 21 mg per 100 ml for comparison. On the other hand, low density and high density lipoprotein concentrations appeared to be somewhat decreased.

All the patients were instructed in a diet designed to bring them to ideal weight and maintain them on a daily intake providing approximately 40 percent of calories from

Table 1

	Cholesterol	Triglycerides	High Density Lipoproteins	Low Density Lipoproteins	Very Low Density Lipoproteins
milligrams per 100 ml					
Men	440	694	37	113	268
Women	470	705	39	131	307

carbohydrate and fat, and 20 percent from protein. In 25 subjects, a weight loss occurred during the early period of negative calorie balance. A striking response of plasma lipids and lipoproteins was noted after dietary therapy. In 12 subjects, clofibrate was required along with the diet therapy. Again, an excellent response was noted (see Table 2). In one subject treated by diet alone whose plasma triglycerides were 2060 mg per 100 ml, a normal value of 160 mg per 100 ml was recorded. Cholesterol in the same subject also decreased from 632 to 174 mg per 100 ml with use of only diet therapy.

The most common form of lipid deposition was xanthoma striata palmaris. Xanthoma striata palmaris consists of unusual yellow deposits in palmar creases. Sixty-four percent of the patients demonstrated this lesion. The incidence of vascular disease was high; some 40 percent of the patients had detectable disease. About one-third of the patients had ischemic heart disease and one-third had peripheral vascular disease; 22 percent of the subjects had both of these diseases. Five patients had episodes of cerebral vascular insufficiency. Ischemic heart disease was detected earlier in men than in women.

Hyperuricemia was present in 40 percent of the subjects and 55 percent had abnormal glucose tolerance tests. Only two patients, however, had fasting hyperglycemia. Neither patient required insulin or was known to develop ketosis.

Body mass was defined as a body mass index and it was noted that this index, approximately 28, was higher than the

average index for the general population which is in the region of 25.

All of the subjects had normal blood urea nitrogen and serum creatinine, alkaline phosphatase, glutamic oxalacetic transaminase, glutamic pyruvate transaminase, bilirubin, calcium, phosphorus, and electrolyte concentrations. Protein electrophoresis was normal. All patients had normal hematocrits and normal thyroid function tests except for two subjects with thyroid dysfunction. Many of the subjects had concomitant diseases not related to hyperlipoproteinemia which included hypertension, nephrolithiasis, gout, peptic ulcer, diabetes mellitus, and asymmetric septal hypertrophy. Others had leukopenia, factors 9 through 11 deficiency, Von Willebrand's disease, and benign monoclonal gammopathy. Six subjects had cholelithiasis, one patient had hypothyroidism, and another had hyperthyroidism. The age of onset of vascular disease in the Type III disease was 38 for men and 50 for women, peripheral vascular disease 38 and 50, and cerebral vascular disease 58 and 54, respectively.

In the relatives, 49 of 107 had hyperlipidemia; an additional 14 had triglycerides greater than 150 although below the 90 percent cut-off limit for study. An additional 17 adult relatives had Type IV disease. Five others had abnormal low density lipoprotein concentrations, three of which had hyperglyceridemia, and two with normal triglycerides. The prevalence of Type III disease in the relatives was 25 percent. Consanguinity was established in only one kindred.

Table 2

	Cholesterol	Triglycerides	High Density Lipoproteins	Low Density Lipoproteins	Very Low Density Lipoproteins
	milligrams per 100 ml				
Diet Therapy	185	131	40	96	50
Diet Therapy + Clofibrate	170	123	44	88	39

Type III disease is seen primarily in adults and this was true in this study. There is only one report of such a case in a child; an obese 10-year-old boy who had xanthomas and hyperlipidemia. Usually xanthomas or atherosclerotic vascular disease is the reason Type III patients consult a physician.

In this study 81 percent of the subjects and 42 percent of the unsuspected Type III relatives had xanthomas. After therapy, xanthomas regressed uniformly, but disappearance of all the cutaneous lesions has not occurred with the use of diet or diet and drug therapy. The diagnosis is strengthened if abnormal glucose tolerance test and hyperuricemia is noted, because both of these findings are present in between 33 and 43 percent of the subjects. Intermittent claudication in a relatively young man or woman with hyperlipidemia should lead one to suspect Type III hyperlipoproteinemia.

These authors note that hypocaloric conditions and achievement of ideal body weight markedly lower plasma lipid levels.

Nutrition therapy is the single most effective means to control hyperlipoproteinemia. In the maintenance diet of 40 percent carbohydrate, 40 percent fat, and 20 percent protein, a polyunsaturated to saturated fatty acid ratio of 2:1 is suggested, with restriction of cholesterol and alcohol. A striking feature of patients with Type III is the tendency to demonstrate marked swings in plasma lipid concentrations from week to week, apparently reflecting sensitivity to dietary manipulation and carbohydrate induction. Hypocaloric conditions markedly lower plasma lipid levels and the achievement of ideal body weight appears to be the most effective aspect of therapy. The importance of each of the components of the recommended diet have not yet been rigidly demonstrated. □

-
1. J. Morganroth, R. I. Levy, and D. S. Fredrickson: The Biochemical, Clinical, and Genetic Features of Type III Hyperlipoproteinemia. *Ann. Int. Med.* 82: 158-174, 1975

NUTRITION CLASSICS

THE Journal of State Medicine

VOLUME XX: 341-368, 1912

THE ETIOLOGY OF THE DEFICIENCY DISEASES.

BERI-BERI, POLYNEURITIS IN BIRDS, EPIDEMIC DROPSY, SCURVY,
EXPERIMENTAL SCURVY IN ANIMALS, INFANTILE SCURVY,
SHIP BERI-BERI, PELLAGRA.

BY
CASIMIR FUNK, Ph.D.

THE diseases mentioned above present certain general characters which justify their inclusion in one group, called deficiency diseases. They were considered for years either as intoxications by food or as infectious diseases, and twenty years of experimental work were necessary to show that diseases occur which are caused by a deficiency of some essential substances in the food. Although this view is not yet generally accepted, there is now sufficient evidence to convince everybody of its truth, if the trouble be taken to follow step by step the development of our knowledge on this subject. This article is written with the intention of giving a summary of the modern investigations, and by means of a careful selection of references to facilitate the research for anybody who wishes to read the original literature. This careful selection was absolutely necessary, for there is perhaps no other subject in medicine where so many contradictory and inexact statements were made, which instead of advancing the research retarded it by leading investigators in a wrong direction.

SEP 1975

All these diseases present some general characters, which may be sketched here. The most prominent symptoms are a general cachexia with an enormous loss of weight; marked nervous symptoms are often present, which are due probably to the degeneration of the peripheral nervous system. It is now known that all these diseases, with the exception of pellagra, can be prevented and cured by the addition of certain preventive substances; the deficient substances, which are of the nature of organic bases, we will call "vitamines"; and we will speak of a beri-beri or scurvy vitamine, which means a substance preventing the special disease. As regards the classification two different groups present themselves: the beri-beri group and the scurvy group. The investigations made on pellagra, however, have not yet resulted in a sufficient elucidation of its etiology to establish it as a deficiency disease and it is included here provisionally owing to its similarity in some respects to the other diseases mentioned.

• • •

THE RESULTS OF OUR KNOWLEDGE OF DEFICIENCY DISEASES APPLIED TO ANIMAL METABOLISM AND NUTRITION.

The results of modern investigation of deficiency diseases seem to be unknown to most physiologists. I noticed only one *résumé* which draws attention to the deficiency diseases with regard to general metabolism; this is the *résumé* of Mandel on protein metabolism [88]. The food was up to now valued only by its content in proteins, fats and carbohydrates, and calories value.

• • •

From the present *résumé* we can conclude that all the deficiency diseases can be prevented by a complete diet. A monotonous diet ought to be avoided, because in this case a deficient food is made use of for long periods and prepares the ground for the outbreak of the deficiency diseases. There is no doubt that as our knowledge of the relative value of different foodstuffs increases we will be able to prevent completely the outbreak of the latter.

MALNUTRITION, SALIVARY VOLUME AND
PROTEIN CONCENTRATION

Protein deficiency in rats during the nursing period resulted in a low rate of saliva production with a reduced total protein secretion by the salivary glands although the actual protein concentration was increased.

Key Words: protein deficiency, salivary gland development, saliva production, salivary protein secretion

The influence of marginal protein deficiency in rats on the development of their teeth, on their salivary glands, and on their susceptibility to dental caries, even after the protein deficiency was stopped, has been the subject of active interest over the past decade or so.¹ The reason for the sharp increase in caries susceptibility as a result of relatively brief periods of marginal protein deficiency has not been defined. In the search for an explanation of this phenomenon, Menaker and Navia studied the effect of protein deficiency during lactation on the volume and protein concentration of whole saliva.² Two groups of female rats were maintained on an otherwise nutritious diet containing either 8 or 25 percent protein from the fifth day of pregnancy until their pups were weaned at 19 days postnatally.

All litters were equalized at eight in order to exert the same strain upon each mother, for the profound influence that litter size has upon body weight of the offspring has been demonstrated repeatedly. For example, Madeira and his co-workers compared the rate of gain in body weight and the rate of eruption of the incisors in ten litters with two pups each, in ten litters with six pups each, and in seven litters with 12 pups each.³ The litters were weaned at 30 days of age. The average increase in body weight was significantly reduced as the litter size increased. The rate of incisor eruption was slightly, but not significantly,

slower among the rats 12 to a litter than among those two to the litter at 25 to 27 days of age. The reduction in rate of incisor eruption was observed again in the large litters at 44 to 46 days of age, although the litters had been weaned two weeks earlier; at this age the reduction was statistically significant.

After weaning at 19 days of age, in Menaker and Navia's experiment, the rats from both the normal and protein-deficient dietary groups were maintained on an adequate diet containing 20 percent lactalbumin.² Saliva was collected over a one hour period after pilocarpine stimulation on the 16th, 24th, and 35th days of age. The volume of saliva and the protein concentration were determined. From these data, the absolute amount of protein secreted was calculated. Rats were sacrificed at 16, 24, and 35 days of age. The submandibular glands of rats which had not been salivated were removed and their wet weights determined at each age interval. Caries evaluations were made for the rats sacrificed at 35 days of age.

At birth the pups of the mothers on the protein-deficient diet were only slightly smaller than those born to mothers receiving the diet containing 25 percent protein. By weaning at 19 days of age, however, the differences in body weight were striking. Reading from the graph as the only source of data on weight, the deficient offspring weighed about 20 g in comparison to 45 g for the weanlings of the control mothers. Although the rats from the deficient mothers were transferred to

the diet with adequate protein at weaning, the gap in body weight was never closed, so that at 35 days of age the rats with the deficient background weighed about 45 g in comparison with 100 g for those with the normal protein background. In other words, the experimental pups grew less than half as rapidly over the 16-day experimental period after weaning as the control pups.

The wet weights of the submandibular glands from the deficient pups were much lower at all three ages than for the normal pups. The ratios of average gland weight for the deficient rats to the average gland weight for the normal rats were 40.2, 39.0, and 55.4 percent for the 19, 24, and 35-day-old rats, respectively. These reduced weights for the glands from the protein-deprived rats are striking and statistically significant. As described in the preceding paragraph, however, the body weight differences between the deficient and normal rats are similarly striking. For example, reading again from the graph, the ratios of average body weight of deficient pups to the average body weight of control pups are around 43 to 45 percent for the three ages. In other words, the growth of the salivary glands has not been preferentially penalized; indeed, the values for the 35-day-old rats suggest that the deficient rats at this age have slightly larger submandibular glands in relation to their body weights than do the controls.

The volume of saliva produced under pilocarpine stimulation was very much less in the deficient rats at all three ages than among those with an adequate dietary protein background. The volume for the deficient rats was consistently only about one-fifth that of the controls; in both populations from deficient and normal mothers, the volume increased about seven-fold from the 16th to the 35th days. The reductions in saliva volume among the rats from protein-deficient mothers are statistically significant at the three ages. Even when the data were taken one step further than by the authors and expressed in terms of volume of saliva per milligram of gland, the

rate of saliva production by the deficient rats was only about one-half that in the control rats.

The protein concentration in whole saliva was higher at all three ages in the rats from deficient mothers than in the control rats. These increases were statistically significant. However, when the total protein in the saliva was calculated by multiplying the volume of saliva by the protein concentration, the absolute amount of protein secreted by the protein-deprived rats was significantly less at all three ages than for the controls. The reduction in protein secreted was about three-quarters for each of the three age groups. The fact that the 35-day-old rats from deficient mothers had had the adequate protein diet for 16 days had no effect on the absolute amount of protein secreted. When related to either gland weight or body weight, this very striking reduction in protein secretion means that the glands of the deficient rats secreted less than half the protein on a gland weight basis and the mouths of these rats were bathed with less than half the salivary protein of normal rats. An interesting project would be to examine whether any of the proteins such as the secretory immunoglobulins were especially affected.

The caries scores for the deficient rats at 35 days of age were much higher than for the controls ($p < 0.001$). This observation corroborates again those from several similar studies conducted over the past decade. The authors suggest that, in part at least, the observed changes in volume and protein concentration of the saliva contributed to the increased caries activity. This hypothesis is entirely reasonable in view of the massive literature demonstrating increased caries activity in experimental animals as a result of surgical removal of one pair or all major glands and rampant caries in man in the case of congenital absence of one or more salivary glands, surgical removal, irradiation damage, or diminished flow due to Sjogren's disease.

It is worth emphasizing that although the retardation in the growth of the young

rats was produced by feeding a protein-deficient diet to the mothers, it is probably inappropriate to attribute this to protein deficiency in the young animals. The limitation of protein in lactating animals probably does not produce a protein-deficient milk but rather produces less milk. Thus the result in the young animals is similar to that produced by large litters compared to small litters rather than protein deficiency per se in the young animals. □

1. Protein Deficiency and Tooth and Salivary Gland Development. *Nutrition Reviews* 32: 24-27, 1974
2. L. Menaker and J. M. Navia: Effect of Under-nutrition During the Perinatal Period on Caries Development in the Rat. V. Changes in Whole Saliva Volume and Protein Content. *J. Dent. Res.* 53: 592-597, 1974
3. M. C. Madeira, S. Hetem, and M. A. Rulli: Relationship Between the Number of Rat Littermates per Dam and Mandibular Incisor Growth. *J. Dent. Res.* 53: 634-636, 1974

ADAPTATION TO LOW PROTEIN INTAKES

In rats urea nitrogen changes rapidly in response to the protein intake. A most striking phenomenon is that key hepatic enzymes involved in nitrogen disposition change in concert and to similar degrees in response to the alterations in diet.

Key Words: urea, urinary nitrogen, enzymes, casein, protein intake, half-lives

It is well accepted that nitrogen output falls as intake is reduced, but the mechanisms underlying this simple but fundamental process are poorly understood. It is known that the major component of urinary nitrogen is usually urea. It has also been established that in malnourished children urea as a fraction of urinary nitrogen is depressed.² The rate at which these changes take place is not clear and an investigation of the mechanisms involved cannot be obtained with human subjects. It has been shown that when the protein intake is markedly reduced the urinary excretion stabilizes in adult men after approximately six to ten days³ and in the infant in approximately two days.⁴

Das and Waterlow⁵ used young rats, altered their nitrogen intake, and studied the nitrogen output and the changes in hepatic enzymes which are concerned with urea metabolism directly or indirectly. The enzymes they studied were the urea cycle enzymes arginase, argininosuccinate lyase, and argininosuccinate synthetase as well as glutamate dehydrogenase and two transaminases, alanine aminotransferase, and aspartate aminotransferase. The last three were chosen because they make amino groups available for entry into the urea cycle.

In the first major experiment, the effect of reducing the casein content of the diet was studied. Rats were changed from a high (135 g of casein per kilogram) to a low (45 g of casein per kilogram) protein diet and urine collected in six hourly periods. It was found that urinary nitrogen reached the level appropriate for the low intake after 24 to 30 hours. Rats were sacrificed at appropriate intervals and the six hepatic enzymes which originally showed a high activity on the high protein diet, then decreased and reached their new level in about the same time. The striking phenomenon was that all six enzymes changed at the same rates and to a similar degree. When the lowest activities of the six enzymes were expressed as a percent of the value on the high protein diet, at 30 hours they ranged from 32 to 39.5 percent. In control rats maintained on the low protein diet, the same enzymes were between 30.5 and 37 percent of the control value. It is thus clear that the enzyme levels had adapted to the level appropriate for the specific diet.

The converse experiment was then done. Rats were maintained on the low protein diet for one week, then some were changed to the high protein diet. Again, the urinary nitrogen reached the appropriate plateau in

about 30 hours. Rats were sacrificed in groups at regular intervals after the change to the high protein diet. By 32 hours all six enzymes had almost reached the levels appropriate for that protein intake. The data from these two experiments were combined, and by plotting the rates of change of the total enzyme activity with time, it was possible to calculate the half-lives of the various enzymes. The half-lives were all remarkably similar, and were the same if the calculations were based on the experiments in which the enzyme activities were increasing or decreasing. Since the half-lives were the same, irrespective of whether the enzyme activity was increasing or decreasing, the authors contend that the changes were caused by a change in the rate of synthesis.

To investigate the possibility that it was simply the nitrogen output which affected the enzyme levels, rats were fed gelatin instead of casein to produce an increase in urinary nitrogen. In these experiments, although the urinary nitrogen did increase, the enzyme activities in the liver actually fell slightly. Body weight also appeared to fall. In the converse experiment in which rats were changed from the diet with gelatin to the low protein diet, urinary nitrogen fell and the activity of the liver enzymes did not change.

When nitrogen intake was related to output in rats on the casein diet, there was an excellent linear correlation, with a slope indicating that a quantity of nitrogen was excreted in the urine equivalent to half of the amount ingested. With gelatin there were fewer experiments, but there was still a linear correlation between urinary nitrogen and nitrogen intake.

Therefore, this study shows that young rats can rapidly reduce their urinary nitrogen in response to lowering dietary nitrogen, and within 30 hours a plateau is reached. This provides new information, but it is not as significant as the demonstration that a wide range of enzymes change in concert and at about the same rate in response to dietary manipulations. It is striking that the enzymes measured differ in their intracellular location, and certainly

in the case of the urea cycle enzymes, differ also in basal activity.

As the authors point out, the change in enzyme activity cannot be a reflection simply of an increase in urea output since increasing urea output by feeding gelatin did not cause a rise in the enzyme activity. The fact that urea excretion did change without a change in urea cycle enzymes is taken to indicate that the "potential capacity" of the enzymes is not fully utilized. Caution should be expressed here. The conditions under which the enzyme is assayed *in vitro* obviously may have little relationship to its activity *in vivo* since levels of such factors as substrate, co-factors, and pH will be different.

In those experiments in which the enzyme activities increase, could the effect be a nonspecific one of increased protein intake enhancing protein synthesis generally? In that case several other enzymes should show increases in activity. However, a nonspecific rise in soluble protein appears unlikely. With a change in dietary casein from 40 to 135 g per kilogram, the total hepatic arginase activity rose some four-fold and aspartate aminotransferase rose similarly. It is unlikely that soluble liver protein would increase four-fold.

The authors considered the possibility that hormones might be the signal for the change in enzyme activity and referred to the work of McLean and Gurney⁶ which showed that cortisol increased urea cycle enzymes. Here one should point out that in adrenalectomized rats those authors found that although the urea cycle enzymes changed, their degree of change was variable, and the aspartate aminotransferase activity did not change. Schimke,⁷ however, has proven that steroids are not the signal for the changes in urea cycle enzymes in response to dietary alterations. He showed convincingly that the enzyme proteins in the two dietary states are identical and have identical kinetic characteristics. He also showed that the change in activity is not simply due to presence of activators or absence of inhibitors. There is simply more enzyme protein. A fundamental difference between Schimke's work and the

present one is that the enzyme half-lives are so widely different. Schimke showed a half-life for arginase of about five days,⁸ as compared with eight hours in the present study. There obviously cannot be such a difference in enzyme protein in the two strains of rats. The reason for these differences is not clear, but perhaps relates to the different methods used, especially for calculations of enzyme half-lives.

It must be confessed that there is no clue from this or other studies as to why these enzymes should change in concert. It is hard to believe that there is a single genome for all six enzymes. The pathway for which the single genome theory was proposed with most vigor is gluconeogenesis.⁹ We know now that although some enzymes with widely differing specific activities do change together in response to some stimuli, there are some in the pathway which do not change, and the degree of change of those that do can be widely varied.

It is hoped that the authors will pursue these studies, perhaps measuring the levels of the key intermediates to determine if and how they do change, and measuring other enzymes as well. (Their data on glutamate dehydrogenase are in conflict with other studies.) The problem of the dietary control of enzyme synthesis and degradation is fundamental in a general physiological sense. We should know what signals the shunting of amino acids into the synthetic pathway and not into the pathway for degradation and/or excretion as a product which is normally not reutilizable to any great extent. In situations when protein is present in short supply the form of control could be important for therapeutic reasons. There may be conditions such as

after surgery or during hyperalimentation in which it may be desirable to conserve nitrogen by reducing the amount entering the pathway for degradation. It is not too naive to suggest that if we knew the mechanism of control it might be possible to adjust it. □

1. J. C. Waterlow: The Partition of Nitrogen in the Urine of Malnourished Jamaican Infants. *Am. J. Clin. Nutrition* 12: 235-240, 1963
2. J. M. L. Stephen and J. C. Waterlow: Effect of Malnutrition on Activity of Two Enzymes Concerned with Amino Acid Metabolism in Human Liver. *Lancet* I: 118-119, 1968
3. N. S. Scrimshaw, M. A. Hussein, E. Murray, W. M. Raud, and V. R. Young: Protein Requirements of Man: Variations in Obligatory Urinary and Fecal Nitrogen Losses in Young Men. *J. Nutrition* 102: 1595-1604, 1972
4. H. Chan: Adaptation of Urinary Nitrogen Excretion in Infants to Changes in Protein Intake. *Brit. J. Nutrition* 22: 315-323, 1968
5. T. K. Das and J. C. Waterlow: The Rate of Adaptation of Urea Cycle Enzymes, Aminotransferases and Glutamate Dehydrogenase to Changes in Dietary Protein Intake. *Brit. J. Nutrition* 32: 353-373, 1974
6. P. McLean and M. W. Gurney: Effect of Adrenalectomy and of Growth Hormones on Enzymes Concerned with Urea Synthesis in Rat Liver. *Biochem J.* 87: 96-104, 1963
7. R. T. Schimke: Adaptive Characteristics of Urea Cycle Enzymes in the Rat. *J. Biol. Chem.* 237: 459-468, 1962
8. R. T. Schimke: The Importance of Both Synthesis and Degradation in the Control of Arginase Levels in Rat Liver. *J. Biol. Chem.* 239: 3808-3817, 1964
9. G. Weber, R. L. Singhal, N. B. Stamm, E. A. Fisher, and M. A. Mentendiek: Regulation of Enzymes Involved in Gluconeogenesis. *Adv. Enzyme Reg.* 2: 1-38, 1964

THE INHIBITION OF CELLULAR UPTAKE OF FOLATE BY FOLIC ACID-BINDING PROTEIN

Studies of the uptake of labeled folates by HeLa cells in tissue culture show that folic acid-binding proteins are not transport proteins and make folates less available for cellular uptake.

Key Words: folic acid-binding proteins, pteroyl-glutamic acid, tetrahydrofolic acid, HeLa cells, cellular uptake of folates

Specific binding proteins for folic acid (FABP) have been demonstrated in a number of materials. These appear to be present in both bovine and human milk¹ and in the sera from folic acid deficient patients. Folic acid-binding materials have also been demonstrated in the sera from patients suffering from a number of pathological states and in women taking oral contraceptives.² The identity of the binding agents in these various sources has not been established but they share some common characteristics. These are a rapid association rate and a slow dissociation rate for the binding of pteroyl glutamic acid and preferential binding of oxidized folyl mono- and polyglutamates compared with reduced folates. There appear to be two proteins involved in folate binding, one a beta-globulin with a molecular weight of around 50,000 and another much larger protein. The amounts of FABP in milk are much higher than in serum. It has been suggested that FABP may be a cellular, perhaps membrane-derived protein with the role of regulating cellular uptake, distribution, and storage of the various folate coenzymes.

The true physiological significance of FABP is yet to be established. Waxman and Schreiber³ recently described some studies on the role of these proteins in the cellular uptake of folates.

The uptake of labeled pteroyl glutamic acid (³H PGA) and methyl-tetrahydrofolic acid (³H methyl-THFA) was studied using HeLa cells in monolayer cultures. Some

HeLa cells were adapted to folate deficiency by passage through a folic acid free medium. The fetal calf serum which is usually added to tissue culture media was dialyzed and as used contained less than 1 ng of folate per milliliter.

The uptake of the labeled folates was measured at 37° and at 4°C; ³H teropterin (a folyl polyglutamate) was also studied under the same conditions.

The cells cultured in a normal medium showed an increased uptake of both forms for some three hours with slight evidence of a plateau after one hour. The uptake of ³H methyl-THFA was greater than for ³H PGA at 37°C whereas the uptake of the polyglutamate was virtually zero. Uptake at 4°C was very low and Dilantin (0.1 mg per milliliter) and ethanol (1 percent) had no effect on the uptake.

The folate-depleted cells showed a reduced growth rate. It was only half that of the normal cells after one week and cell death was increased in the folate-deficient cells after three weeks. There was also evidence of deranged DNA synthesis, as judged by the suppression of the incorporation of ³H thymidine by deoxyuridine. DNA synthesis was restored to normal in these cells by the addition of pteroyl glutamic acid.

Uptake of the labeled folates by these depleted cells was much greater, by a factor of five for ³H PGA and three for ³H methyl-THFA compared with the normal cells. There was no difference in the uptake of the two forms.

When the cells were incubated with various substances (normal sera, folate-deficient and uremic sera, cow's milk,

beta-lactoglobulin, and human milk) containing varying amounts of FABP, there was a considerable reduction in the uptake of the two forms of labeled folates compared with cells incubated with normal human albumin. The inhibition of uptake was related to the FABP content of the various test substances as measured by their capacity to bind ^3H PGA.⁴

HeLa cell cultures are rapidly doubling lines so that a large proportion of the cells are actively synthesizing DNA at any one time. One would therefore expect them to have a high requirement for folate.

The temperature dependence of uptake together with the absence of an effect of Dilantin or ethanol suggest that an energy-dependent active transport mechanism is involved in both PGA and methyl-THFA uptake.

The FABP in the various substances and sera tested appeared to make the labeled folates less available for uptake into the HeLa cells. In the sera tested the inhibition of ^3H PGA uptake was directly proportional to their FABP contents. It therefore appears that FABP in the serum will retard the delivery of folates into the cell and that FABP is not a serum-delivery protein for folate. It is possible that under certain conditions serum FABP could produce intracellular folate deficiency despite adequate serum folate levels. This situation has been suggested as the cause of megaloblastic marrow maturation in some uremic patients whose sera contain FABP.

Milk FABP has been shown to depress the bacterial uptake of folate. FABP in the mammary gland may act as a mechanism for accumulating folate from the blood plasma into the milk, and in the gut facilitate absorption by preventing folate uptake by the intestinal microorganisms.⁵ The milk FABP may influence the availability of folate to the neonate as well as influencing the ecology of the gut microflora.

FABP could not be detected in portions of the culture media from the HeLa cells cultured in the absence of folate although it is released into the media by cultures of chronic myelogenous leukemia cells.⁶ FABP has been demonstrated in the brush border cells of rat small intestine and human lymphocytes. These suggest that FABPs are cellular-derived proteins which may be involved in the intracellular accumulation of folate. FABP in the membrane may be the rate limiting factor in folate uptake because it could act as a folate carrier during transport across the membrane, its availability then being determined by the folate state of the cell.

Much remains to be done before the true physiological role of these folic acid-binding proteins is established and the place of these proteins in folic acid metabolism is understood. Folic acid nutrition represents many puzzling features at present and the elucidation of the role of these binding proteins may resolve some of these problems. □

1. J. Ghitis: The Folate Binding in Milk. *Am. J. Clin. Nutrition* 20: 1-4, 1967
2. Folate Binder in Leukocytes and Serum. *Nutrition Reviews* 33: 9-10, 1975
3. S. Waxman and C. Shreiber: The Role of Folic Acid Binding Proteins (FABP) in the Cellular Uptake of Folates. *Proc. Soc. Exp. Biol. Med.* 147: 760-764, 1974
4. S. Waxman and C. Schreiber: Measurement of Serum Folate Levels and Serum Folic Acid-binding Protein by ^3H -PGA Radioassay. *Blood* 42: 281-290, 1973
5. J. E. Ford: Some Observations on the Possible Nutritional Significance of Vitamin B₁₂-Folate-Binding Proteins in Milk. *Brit. J. Nutrition* 31: 243-257, 1974
6. M. da Costa and S. P. Rothenberg: Studies of the Folate Binding Factor in Cultures and Subcellular Fractions of Chronic Myelogenous Leukemia (CML) Cells. *Clin. Res.* 22: 486A, 1974

BISULFITE TOXICITY IN MOLYBDENUM—DEFICIENT RATS

Bisulfite ion is more toxic to rats made molybdenum deficient by a combination of a low molybdenum diet and treatment with tungsten than it is to normal animals.

Key Words: molybdenum, bisulfite toxicity, sulfur-dioxide toxicity

The effect of nutritional state on the susceptibility of experimental animals and man to various toxic substances is a newly emerging and important area of nutrition. A case in point is the recent report of the increased susceptibility of molybdenum-deficient rats to the toxic effects of the important environmental contaminant SO_2 and to bisulfite ion, an ionized form of the hydration product of SO_2 .¹

The enzyme sulfite oxidase (EC 1.8.3.1) catalyzes the oxidation of sulfite to sulfate. It has been purified from the livers of various animals, including the rat,² and has been shown to contain molybdenum. It is postulated that the physiologic function of this enzyme is to oxidize sulfite ions resulting from the metabolism of sulfur amino acids. A case of deficiency of this enzyme in humans has been reported.^{3,4} This child excreted no sulfate in his urine. He also exhibited severe neurologic defects. It is possible to produce a deficiency of sulfite oxidase activity in experimental animals by feeding a low molybdenum diet (30 μg per kilogram of diet) and allowing the animals free access to deionized water supplemented with 100 ppm tungsten as sodium tungstate.¹ Tungsten has been shown to be a competitive antagonist of molybdenum in animal systems.^{2,5}

It has recently been reported that sulfite oxidase is involved in regulating the toxicity of SO_2 and bisulfite.¹ In these studies rats were fed a low molybdenum diet and allowed access to deionized water supplemented with tungsten. After three weeks on this diet, the hepatic sulfite oxidase activity and liver molybdenum content were about 10 percent and 5 percent, re-

spectively, those of controls fed the low molybdenum diet but without tungsten. The activity of xanthine oxidase, another molybdenum requiring enzyme was also markedly reduced. Otherwise, the animals appeared healthy. The tungsten treatment had no effect on body or liver weight.

After three to five weeks on the diet the LD_{50} of sodium bisulfite, administered by intraperitoneal injection, was determined. The animals deficient in sulfite oxidase were found to be more sensitive to bisulfite toxicity than controls. The median lethal dose of NaHSO_3 in rats maintained on the low molybdenum diet plus tungsten for three weeks and five weeks were 271 and 181 mg per kilogram, respectively. The median lethal dose for animals maintained on rat chow was 551 mg per kilogram and on the low molybdenum diet without tungsten (control animals) was 475 mg per kilogram. In all groups, death was preceded by prostration and seizures, indicating that bisulfite was affecting the central nervous system. It had been suggested previously that the site of toxicity of bisulfite was the central nervous system.⁶ These data indicated that the ability of the animals to survive was dependent upon how rapidly they could metabolize bisulfite to sulfate.

There was no difference in the survival rate of control animals and those deficient in sulfite oxidase when exposed to different levels of SO_2 . At certain levels of SO_2 exposure, however, there was a difference in survival times. At a high level (50,000 ppm) death occurred rapidly (less than 10 minutes) with no difference in survival times between the two groups. The symptoms of toxicity at this level of exposure were indicative of an effect on the central nervous system as in the case of bi-

sulfite ion. At 925 ppm the animals deficient in sulfite oxidase exhibited a much shorter survival time than the control group. In addition, the symptoms accompanying death were different. The animals deficient in sulfite oxidase again exhibited symptoms indicating primarily central nervous system toxicity while the control animals showed symptoms indicating largely respiratory difficulties. Again, the symptoms seen in the sulfite oxidase deficient group were similar to those exhibited by all groups following bisulfite intoxication. At 590 ppm of SO_2 only symptoms of respiratory difficulties were seen in both groups. These data suggest that sulfite oxidase reduces the systemic toxicity of SO_2 but appears to play no significant role in reducing the subacute and chronic respiratory effects of this gas. An explanation for the difference in survival times of sulfite oxidase deficient as compared to control animals exposed to SO_2 may be related to rate of oxidation of bisulfite ion formed from SO_2 in vivo. It is proposed that at high doses of SO_2 the bisulfite levels are too high and are attained too rapidly in both the control and sulfite-oxidase deficient groups for a protective effect of the enzyme against the central nervous system effects to be possible. At an intermediate exposure level (925 ppm) the bisulfite ion formed in controls is detoxified so that respiratory symptoms of toxicity predominate, while in the sulfite-oxidase deficient animals the central nervous system toxicity of the bisulfite ion is the principal cause of death. At lower levels of SO_2 (500 ppm) insufficient bisulfite ion is formed and both groups succumbed to the respiratory effects of the gas.

The sulfite oxidase levels in human lung and liver are comparable to those in the

corresponding organs in rats.¹ Normal rats exhibit no symptoms of systemic poisoning from SO_2 at 1,000 ppm. Thus, at atmospheric concentrations which rarely exceed 5 ppm, present SO_2 levels in urban environments do not appear to pose any systemic hazard. Any noxious effects of SO_2 at this level will most probably result from exposure of the epithelial tissues in the lung to the gas. The enzyme sulfite oxidase does not appear to protect the animal to any significant degree against this effect of SO_2 . □

1. H. J. Cohen, R. T. Drew, J. L. Johnson, and K. V. Rajagopalan: Molecular Basis of the Biological Function of Molybdenum. The Relationship between Sulfide Oxidase and the Acute Toxicity of Bisulfite and SO_2 . *Proc. Nat. Acad. Sci. USA* 70: 3655-3659, 1973
2. J. L. Johnson, H. J. Cohen, and K. V. Rajagopalan: Molecular Basis of the Biological Function of Molybdenum. Molybdenum-Free Sulfite Oxidase from Livers of Tungsten-Treated Rats. *J. Biol. Chem.* 249: 5046-5055, 1974
3. F. Irreverre, S. H. Mudd, W. D. Heizer, and L. Laster: Sulfite Oxidase Deficiency: Studies of a Patient with Mental Retardation, Dislocated Lenses and Abnormal Urinary Excretion of S-sulfo-L-cysteine, Sulfite and Thiosulfate. *Biochem. Med.* 1: 187-217, 1967
4. S. H. Mudd, F. Irreverre, and L. Laster: Sulfite Oxidase Deficiency in Man: Demonstration of the Enzymatic Defect. *Science* 156: 1599-1602, 1967
5. E. S. Higgins, D. A. Richert, and W. W. Westerfeld: Molybdenum Deficiency and Tungstate Inhibition Studies. *J. Nutrition* 59: 539-559, 1956
6. J. W. Wilkins, J. A. Green, Jr., and J. M. Weller: The Toxicity of Intraperitoneal Bisulfite. *Clin. Pharmacol. Therap.* 9: 328-332, 1968

EFFECTS OF ORAL AND PARENTERAL FEEDING ON PANCREATIC ENZYME CONTENTS

The end products of carbohydrate and fat digestion, glucose, and free fatty acids, stimulate increased quantities of amylase and lipase in the homogenized pancreas. Amino acids given orally or intravenously do not increase protease concentrations nor does protein when given orally.

Key Words: insulin, pancreas, enzymes, lipids, protease

Total parenteral nutrition has increased in its usage as a reliable and useful method in the treatment of serious nutritional problems in human beings. The absence of food circulating through the intestinal tract for long periods, however, has the possibility of producing complications such as gallstones because of no stimuli for biliary secretion. Other effects on gastric, intestinal, and pancreatic functions are presently unknown. The pancreas reacts to total parenteral nutrition in its endocrine function; the great inflow of glucose by the intravenous route stimulates increased insulin production. When total parenteral therapy is stopped suddenly, insulin continues to be secreted at an increased rate and hypoglycemia can result. Recently, a study of the exocrine function of the pancreas has been made and the effects of oral and parenteral feedings on pancreatic enzymes were determined in the rat.¹ The primary reason for the study was not in regard to total parenteral nutrition, but certainly such relationships can be made, keeping in light the fact that animal studies cannot always be directly related to human beings. Adaptation of the pancreas by regulating exocrine enzyme secretions in relation to the products of digestion was determined. If the pancreas detects the composition of the diet through products of digestion coming through the blood, then there should be no difference in the levels of enzymes within the pancreatic tissue if the major components of the diet, such as

carbohydrate, lipid, and protein, are given via intravenous or oral routes.

Three types of powder form diets were fed: a high fat diet which was either high lard or high oleic acid, a high casein or a high amino acid diet, and a high starch or high glucose diet. In the parenteral nutrition diets, glucose was the solution for the carbohydrate test, a soy bean emulsification preparation was given for the lipid parenteral nutrition, and an amino acid solution was given for the protein experiments. The pancreas was homogenized and the levels of different enzymes in the pancreas determined.

The studies were not, unfortunately, clear in that both oral and parenteral feedings were given simultaneously. However, when glucose was given intravenously a high fat diet was allowed, when fat was given intravenously a high starch diet was fed, and when amino acids were given intravenously a high starch diet was allowed. When rats were given parenteral nutrition, a reduction in oral intake was observed. Some rats stopped all oral intake and lost weight. Only those animals which did not lose weight during infusion were used for the study.

When large amounts of carbohydrate were infused as glucose in the vein or fed as a high glucose or a high starch diet, the specific activity of amylase was three times greater than that of controls fed a high lard diet. Carbohydrates enhanced pancreatic amylase levels whether the route of administration was via oral or intravenous routes. It made no difference as to the degree of hydrolysis required before

glucose was absorbed. All animals demonstrated a drop of pancreatic lipase concomitant with a reduction in fat intake. Proteolytic enzyme levels were not affected by the nutritional manipulations.

When lipid was given intravenously or via the oral route, a rise in pancreatic lipase level occurred. A high oleic acid diet had the same effect on lipase as did the high lard diet. Thus, lipids, like carbohydrates, increased pancreatic lipase no matter what route they were given. It also made no difference as to the amount of hydrolysis of the fat which was required before absorption occurred.

In the protein experiments, however, it was only via the oral route that there was a noticeable increase in trypsinogen, chymotrypsinogen, and lipase in the pancreatic tissue. When amino acids were fed orally or given intravenously, there was no effect on these enzyme levels. Lipase decreased under these conditions.

It was apparent, therefore, that the pancreas adapts to dietary changes not only depending on the substance undergoing digestion, but also in the case of carbohydrate and fat, to the end products of digestion.

It was concluded that products of hydrolysis of carbohydrate and lipid, although not requiring pancreatic enzymes for absorption, are capable of inducing specific pancreatic enzymes. These end products of digestion, therefore, must act after their passage into the blood. The studies of amino acids, however, were in sharp contrast to these data and indicate that the enzyme adaptation is different in the case of protein metabolism. Protein must be in the gut to induce increased quantities of proteases in the pancreas. These data indicate that it may be useful to feed protein occasionally to people on long-term total parenteral nutrition to maintain a given level of proteases within their pancreas. Lipid feedings have been advocated from time to time in such patients to maintain gallbladder contractions. The two might be easily combined to prevent any problems with disuse atrophy which might occur over long periods of therapy. □

-
1. M. Lavau, R. Bazin, and J. Herzog: Comparative Effects of Oral and Parenteral Feeding on Pancreatic Enzymes in the Rat. *J. Nutrition* 104: 1432-1437, 1974

Copper Metabolism in the Steely-Hair Syndrome

Key Words: kinky-hair syndrome, steely-hair syndrome, copper metabolism, copper deficiency

Menkes and co-workers¹ described (in 1962) children with developmental regression, seizures, temperature instability, scorbutic-like bone changes, and a peculiar steel-like hair. Danks and his co-workers² reported a defect in copper absorption.

Lott et al.³ investigated the copper metabolism in an 11-month-old male with this syndrome. Serum copper was 15 μ g per 100 ml and serum ceruloplasmin undetectable. Less than 1 percent of orally administered ⁶⁴Cu was absorbed. This patient was given supplemental copper as cupric sulfate in two divided doses totalling 0.52 mg per kilogram per day. Within seven days of such supplemental oral copper, the serum copper rose three-fold (from 15 μ g per 100 ml) and remained at approximately 75 percent of the normal concentration for the remaining 22 days of therapy. After the end of supplementation, the serum copper returned to baseline in about ten days. Al-

though no oxidase activity could be found indicating ceruloplasmin, all samples did contain an immunoprecipitation band, although in a reduced amount. It is concluded that the copper absorption block is only partial in the Steely-hair syndrome and is overcome by high doses of oral supplements. □

1. J. H. Menkes, M. Alter, G. K. Steigleder, D. R. Weakley, and J. H. Sung: A Sex-Linked Recessive Disorder with Retardation of Growth, Peculiar Hair, and Focal Cerebral and Cerebellar Degeneration. *Pediatrics* 29: 764-779, 1962
2. D. M. Danks, P. E. Campbell, B. H. Stevens, V. Mayne, and E. Cartwright: Menkes Kinky Hair Syndrome—An Inherited Defect in Copper Absorption with Widespread Effects. *Pediatrics* 50: 188-201, 1972
3. I. T. Lott, R. DiPaolo, D. Schwartz, S. Janowski, and J. N. Kanfer: Copper Metabolism in the Steely-Hair Syndrome. *New Engl. J. Med.* 292: 197-199, 1975

Alpha-1-Antitrypsin Deficiency—Liver Disease

Key Words: cirrhosis, sialic acid, cell transport, alpha-1-antitrypsin, antitrypsin sialyltransferase

Eriksson and Larsson¹ purified the PAS-positive inclusion bodies from cases of cirrhosis associated with homozygous alpha-1-antitrypsin deficiency. The main component was a protein of the same molecular size as the serum alpha-1-antitrypsin and has immunological similarity. Chemical analysis of this liver material showed a

complete absence of sialic acid, as a major difference with the normal serum protein.

It is now established that deficiency of serum alpha-1-antitrypsin is associated with some cases of chronic obstructive pulmonary disease and some cases of fatal cirrhosis in the perinatal or juvenile period. More recently, hepatocellular carcinoma has been associated with a partial deficiency.² Although the pulmonary damage is postulated to represent the uninhibited

proteases of bacterial and leukocytic origin in the lung, the liver damage is believed in some way related to the accumulation of the PAS-positive material in the liver cells. The present authors indicate that the PAS-positive material is immunologically similar to serum alpha-1-antitrypsin, but is different in lacking a terminal sialic acid moiety found in the serum protein. This suggests that there is an inability to add sialic acid to the precursor.

Kuhlenschmidt³ suggested that this inability to add sialic acid is due to a deficiency of a sialyltransferase. They studied one patient with antitrypsin deficiency and juvenile cirrhosis and demonstrated that the PAS-positive material obtained by liver biopsy did not stain for sialic acid; furthermore they found decreased serum sialyltransferase activity in the patient.

Together these observations suggest that there is a deficiency of sialyltransferase and

the accumulation of the unsialyated material in the liver indicates that this is a defect in cellular export. The accumulation in some manner is responsible for the liver disease. □

1. S. Eriksson and C. Larrson: Purification and Partial Characterization of PAS-Positive Inclusion Bodies from the Liver in Alpha₁-Antitrypsin Deficiency. *New Engl. J. Med.* 292: 176-180, 1975
2. W. Rawlings, J. Moss, H. S. Cooper, and S. R. Hamilton: Hepatocellular Carcinoma and Partial Deficiency of Alpha-1-Antitrypsin (MZ). *Ann. Int. Med.* 81: 771-773, 1974
3. M. S. Kuhlenschmidt, E. J. Yunis, R. M. Iammarino, S. J. Turco, S. P. Peters, and R. H. Glew: Demonstration of Sialyltransferase Deficiency in the Serum of a Patient with Alpha-1-Antitrypsin Deficiency and Hepatic Cirrhosis. *Lab. Invest.* 31: 413-419, 1974

Recent Publication

National Center for Health Statistics. *Hematocrit Values of Youths 12 to 17 Years, United States*. VITAL AND HEALTH STATISTICS, Series 11, No. 146, DHEW Publication No. (HRA) 75-1628, Pp. 40. Health Resources Administration, DHEW, Rockville, Maryland 20852.

This report from the National Center for Health Statistics presents the first of the findings from the sample of blood drawn from each of 6,768 youths examined during Cycle III of the Health Examination Survey.

Blood hematocrit values (the volume occupied by the red blood cells contained in 100 ml of blood) were obtained from venous blood samples. These samples were taken from each examinee during the course of the three and one-half hour examination administered by the Health Examination Survey to a probability sample of the country's civilian, noninsti-

tutionalized population aged 12 to 17 years. Determination of the blood hematocrit values was made by the micro-method.

The hematocrit value is determined by centrifuging a standard amount of whole blood (100 ml) at 12,500 r.p.m. for five minutes. The hematocrit value gives information on the hematological status of a subject, and it is currently the simplest and single most accurate measurement of anemia or polycythemia available to the health profession.

In male youths, mean hematocrit levels increased consistently with age from a low of 40.5 ml percent in 12-year-olds to a high of 45.8 ml percent in 17-year-olds. No such increase with age was seen in females. The increase with age in mean hematocrit found for male youths in the United States was also found for Negro and white males separately. White female youths showed no increase with age, and at each age, Negro

females had lower mean hematocrit levels than white females.

A small increase in mean hematocrit occurred with increase in annual family income for both male and female youths. An increase in mean hematocrit was also found with an increase in the education of the parent. These two findings held for white youths, when examined separately, but did not hold for Negro youths.

Variables that influence hematocrit values and relevant studies in the literature are discussed.

Twenty tables present detailed statistics on percentiles, means, standard deviations, and standard errors of the mean for hematocrit by age, race, sex, geographic region, annual family income, and education of parent. Three appendixes present statistical notes, demographic variables, and techniques of measurement and quality control.

Single copies of this publication are available free of charge from NCHS, Room 8-20, 5600 Fishers Lane, Rockville, Maryland 20852, Attn: M. Flaer. Multiple copies may be purchased for \$1.00 each (prepaid) from the Superintendent of Documents, U.S. Government Printing Office, Washington, D.C. 20402. □

Letter to the Editor

Milk Composition Questioned

Sir: With great interest I read the article on lactation and composition of milk in undernourished women (*Nutrition Reviews* 33:42-43, 1975).

I did wonder, however, why the references were restricted to the protein, fat, and lactose contents.

In contradiction with these nutrients, the levels of vitamins in human milk is often very low when the mothers consume vitamin-poor diets.

For vitamin A this was already shown as early as 1938 by Dutch workers in Java.¹

It was claimed that "the mothers make their children blind with their milk."

Trusting this information will be of some use to you.

Dr. R. Luyken, Professor
Central Institute for Nutrition
and Food Research
Utrechtseweg 48, Zeist
Netherlands

-
1. J. H. de Haas and O. Meulemans, *Tijdschr. Nederl. Indie* 78: 847-855, 1938

Recent Books

The Liver: Normal and Abnormal Functions. Edited by F. F. Becker. Published by Marcel Dekker, Inc., 270 Madison Avenue, New York, New York 10016. Pp. 423. Price \$37.50.

Total Parenteral Nutrition: Premises and Promises. Edited by H. Ghadimi. Published by John Wiley and Sons, New York. Pp. 632. Price \$29.50.

High-Quality Protein Maize. Proceedings of the CIMMYT-Purdue Symposium on Protein Quality in Maize. Edited by L. F. Bauman, E. T. Mertz, A. Carballo and E. W. Sprague. Published by Dowden, Hutchinson & Ross, Inc., Stroudsburg, Pennsylvania. Distributed by Halsted Press. Price \$28.00.

New from Academic Press

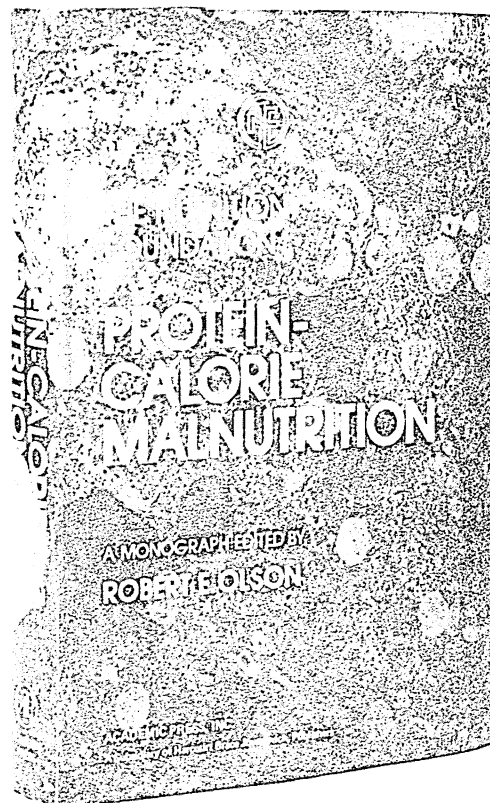
PROTEIN-CALORIE MALNUTRITION

A Nutrition Foundation Monograph

For physicians, nutritionists, public health practitioners, social workers, and medical students.

Covers all aspects of protein-calorie malnutrition (PCM), from molecular biology to epidemiology and prevention.

467 pages, \$29.50. Order from:
Academic Press, 111 Fifth Avenue,
New York, New York 10003
or your book supplier.



CONTENTS

Amino Acid Requirements and Age

Adaptation to Low-Protein Intakes

Protein Turnover in Various Nutritional States

Lysine Metabolism in Protein-Calorie Malnutrition with
Attention to the Synthesis of Carnitine

Cellular Energy Metabolism in Protein-Calorie Malnutrition

Brain DNA Synthesis in Protein-Calorie Malnutrition

Electroencephalograms, Learning, and Malnutrition

The Relationship of Severe Marasmic Protein-Energy
Malnutrition and Rehabilitation in Infancy to Subse-
quent Mental Development

Carbohydrate and Lipid Metabolism in Protein-Calorie
Malnutrition

Water-Soluble Vitamins in Protein-Calorie Malnutrition

Nutritional Survey of Children in the Dominican Republic
with Attention to Deficits in Water-Soluble Vitamins

The Fat-Soluble Vitamins and Protein-Calorie (Energy)
Malnutrition

Mineral Metabolism in Protein-Calorie Malnutrition

Mineral Metabolism in Protein Malnutrition

Endocrines and Malnutrition

Erythropoiesis in Protein-Calorie Malnutrition

The Anemia of Protein-Calorie Malnutrition

The Effect of Vitamin E on the Anemia of Protein-Calorie
Malnutrition in Northern Thai Children

The Effect of Variations in Protein and Calorie Intake on
the Rate of Recovery and Selected Physiological Res-
ponses in Thai Children with Protein-Calorie
Malnutrition

The Liver in Protein-Calorie Malnutrition: An Ultrastruc-
tural Study

Calories in the Treatment of Protein-Calorie Malnutrition

Protein-Sparing Effect of Excess Calories during Adapta-
tion to Low-Protein Intake

Protein versus Calories in the Treatment of Protein-Calorie
Malnutrition: Metabolic and Population Studies in India

Interactions of Malnutrition and Infection: Advances in
Understanding

Immunoglobulins and Complement in Protein-Calorie
Malnutrition

Cell-Mediated Immunity in Protein-Calorie Malnutrition

Treatment of Severe Protein-Calorie Malnutrition

The In-Patient and Out-Patient Treatment of the Child
with Severe Protein-Calorie Malnutrition

Prevention of Vitamin and Mineral Deficiencies Associated
with Protein-Calorie Malnutrition

Prevention of Protein-Calorie Malnutrition

28 NOV 1975

Prevention and Therapy of Diabetes Mellitus

by Kelly M. West, M.D.

Diabetes mellitus is a disorder characterized by hyperglycemia, a relative or absolute deficiency of insulin, and by certain characteristic symptoms and pathologic manifestations. Classical symptoms include polyuria, polydipsia, and weight loss, but these occur only with moderate or marked elevations of the blood glucose. They are entirely reversible with treatment. A substantial percentage of untreated diabetics have mild hyperglycemia without these classic symptoms. Both mild and severe diabetics are, however, prone to develop morbid changes in small and large arteries and in nerves. Complete failure of beta-cell function if untreated or inadequately treated, leads to profound hyperglycemia, ketonemia, acidosis, negative nitrogen balance, dehydration, and other severe aberrations in the intermediary metabolism of carbohydrates, lipids, proteins, and electrolytes. Untreated severe diabetes eventually leads to coma and death. Although important nutritional deficiencies, such as muscle wasting and growth failure, occur occasionally in undiscovered or inadequately treated cases of severe diabetes, the availability of insulin therapy has made these uncommon. Today the main challenge is the prevention, miti-

gation, or postponement of the vascular and neurologic manifestations. These include retinopathy and glomerulosclerosis, increased rates of coronary atherosclerosis, and gangrene secondary to arterial insufficiency and impaired sensation in the legs.

Many different factors can produce diabetes or increase risk of the disease; the most important of these are obesity and genetic factors. Diabetes may also be caused or precipitated by any agent that directly impairs beta-cell function or destroys beta cells (e.g., pancreatitis) or by factors that increase peripheral resistance to insulin (e.g., acromegaly or obesity). Adult-onset diabetes is much more frequent than youth-onset diabetes. About three-fourths of adult-onset cases are obese. Only a very small percent of youth-onset cases are obese. Typically, youth-onset diabetes is severe (little or no endogenous insulin); while adult-onset diabetes is usually mild or moderate in severity because beta-cell failure is only partial.

This essay will summarize present knowledge concerning three related aspects of nutrition and diabetes. Discussion will include the effects of nutritional factors on the risk of diabetes, the effect of diet on the manifestations of diabetes, and the role of diet in the treatment of this disorder including some of the specific dietary objectives and strategies in the various types of diabetes.

Nutrition and Etiology

It has long been suspected that nutritional factors affect the risk of diabetes. More recent investigations elucidate further

Dr. West is Professor of Medicine at the Department of Medicine, University of Oklahoma Health Sciences Center, 800 N.E. 13th Street, Oklahoma City, Oklahoma 73190.

A limited number of reprints of this article may be obtained from the author. THERE ARE NO REPRINTS OF UNSIGNED REVIEWS.

the nature and strength of these relationships. Changing dietary patterns in Japan,¹ Israel,² and Africa³ have been associated with a profound increase in the rates of diabetes. Studies by the author with Kalbfleisch and other collaborators in 13 societies of 11 countries indicated a strong relationship of diabetes prevalence and nutritional factors.⁴⁻⁶ A recent review of available data on past and present rates of diabetes in aboriginal populations of the New World (Indians, Eskimos, Polynesians, and Micronesians) also suggested a strong relationship of diet and a risk of diabetes.⁷ Marked differences in nutritional factors and exercise levels in these populations probably account for differences in rates of diabetes as great as ten-fold.⁷

The factor most strongly and consistently associated with prevalence of adult-onset diabetes is the degree and the duration of adiposity. Arguments have also been advanced that dietary sugar^{2,3} and fat⁸ are especially diabetogenic. In this essay, the term "sugar" refers to all mono- and disaccharides but a substantial majority of dietary sugar is usually sucrose in those societies in which sugars furnish as much as 10 percent of the total calories. Increased rates of diabetes have frequently been observed in populations where sugar intake has increased.^{2,3,5,7} It has been difficult, however, to determine whether this is a direct relationship of cause and effect.^{5,7,9} This is because these dietary changes are usually temporally related to other factors such as decreasing exercise, increases in total calories, and fat intake, etc. Moreover, no relationship could be found between the risk of diabetes and the previous sugar consumption when small groups of those with and without diabetes were studied in each of four different populations.¹⁰⁻¹³ In contrast, most intrapopulation studies showed that adiposity is a strong risk factor.^{5,14} Cleave,³ with support from Cohen² and others, argued eloquently that a main precipitating factor in diabetes is the consumption of refined carbohydrates (both sucrose and other "refined" carbohydrates). Keen¹⁵ thought that present evi-

dence was not conclusive in this respect. Although we have found among populations a generally positive association between rates of diabetes and consumption of both sugar and fat, we have also accumulated some epidemiologic evidence that is inconsistent with the hypothesis that sugar and fat are important risk factors apart from their effects or possible effects on levels of caloric consumption.⁴⁻⁷ Hims-worth reviewed evidence that dietary carbohydrate protects against diabetes.⁸ In general, rates of diabetes are low where starch consumption is high.⁶ Trowell recently summarized evidence suggesting that, under certain conditions, the removal of fiber from flour and other foods may enhance the risk of diabetes.¹⁶

The relative importance of sugar in determining the risk of obesity is also not well established.^{9,14} This may vary depending on what foods are available as replacements when sugar intake is intentionally limited or unavailable. Since refined sucrose is a concentrated and an attractive source of calories, it is widely suspected that its consumption would tend to increase the risk of obesity. High rates of obesity have also been observed occasionally in populations in which sugar consumption is low,^{9,14} and in one society, fat people probably ate less sugar than lean persons.¹⁵ High rates of diabetes have not been reported in any society in which obesity is rare. Investigations in the laboratory give considerable support to obesity as a risk factor. For example, obesity is associated with resistance to endogenous insulin.

Cohen produced mild diabetes without producing obesity in one group of rats by feeding high sucrose diets.² These diets, however, were much higher in sucrose (72 percent of calories) than those consumed by any human population. Moreover, obesity and diabetes have also been induced repeatedly in animals by increasing the dietary fat. Under these conditions, the percent of calories as starch or as sugar were often reduced. In one experiment diabetes was induced by a diet high in

protein.¹⁷ Experiments of this kind are often difficult to interpret because two or more variables are usually changed. For example, if fat is increased, it is usually also necessary to decrease carbohydrate or increase calories. In hamsters that were prone to diabetes, Gerritsen and Dulin reduced rates of diabetes dramatically by reducing food intake.¹⁸ This was a quantitative and not a qualitative change in diet. A review of all available laboratory and epidemiologic evidence suggests that the most important dietary factor in increasing the risk of diabetes is total calorie intake irrespective of source. This still leaves open the question of the relative importance of specific nutrients such as fat and sugar in inducing excessive caloric consumption. Previously it was commonly believed that ingestion of refined carbohydrates might "overstrain" the beta cells. Recent physiologic evidence generally tends to diminish this possibility. For example, it has been found that the ingestion of mixed meals containing carbohydrate, fat, and protein stimulate beta-cell function much more strongly than carbohydrate alone.

Severe malnutrition in childhood (as in India and Africa) is sometimes associated with an increased risk of pancreatic calcification and diabetes later in life. Excessive iron consumption may lead to diabetes, secondary to hemochromatosis. Under certain conditions impaired glucose tolerance has been observed with deficiencies of zinc and of chromium, but it is not yet certain whether these deficiencies are significant risk factors for clinical diabetes.

Complications of Diabetes

Among populations of diabetics there are sometimes substantial differences in the frequency of certain complications. In Japan, for example, coronary disease and gangrene are much less common manifestations of diabetes than in the United States. It seems quite probable that the comparatively low rates of atherosclerosis seen in the diabetics in many societies of Asia, Africa, and Latin America are attributable to their diets which are lower in cholesterol

and saturated fat (both before and after discovery of diabetes). Caloric intake is also low in relation to energy expenditure in most of these populations. Geographic and ethnic differences among populations in rates of small-vessel disease (glomerulosclerosis and retinopathy) are considerably less. There are, however, a few societies in which microvascular disease seems to be less frequent in diabetics (e.g., Navajo Indians and Nigerians).^{7,19} Nutritional factors may or may not contribute to these differences. Although present data are not very satisfactory it appears that there are differences among societies in rates of juvenile diabetes.⁷ It is possible that nutritional factors are influential in this respect. In Japan, for instance, marked changes in the national diet during recent years have been associated with substantial increases in rates of juvenile diabetes.²⁰ Rates of ketosis appear to vary among populations of diabetics, but it is not yet clear whether dietary factors play a role in these variations.^{1,7}

Diet Therapy

In recent years considerable progress has been made in understanding the potentialities and priorities of diet therapy in the various types of diabetes. It is now clear that in several respects, dietary objectives and strategies should be quite different in the two main types of diabetes. In maturity-onset obese diabetics, reduction of adiposity by restricting calories or increasing exercise, or both, reduces hyperglycemia. Even more fundamental and more important, these measures also reduce the insulin resistance that attends obesity. This mitigates the "overstrain" of the beta cells and usually leads to considerable improvement of beta-cell function. Thus, in this group, diet not only helps to control diabetes, it reduces the severity of the disease. Return of glucose tolerance to entirely normal levels is uncommon only because of the rarity with which weight is restored to optimum levels and maintained at such levels. However, even partial mitigation of adiposity is helpful. Although most

experts still advise restriction or complete proscription of refined sugars, it is now the consensus that limitation of calories is usually the prime goal of therapy in this type of obese diabetic. In order to establish new eating habits and patterns it may also be desirable to encourage a certain degree of consistency in the quantity, characteristics, and timing of the feedings in obese diabetics. These latter strategies, however, are not of urgent priority in this type of diabetes. For example, a high degree of consistency is not required from day to day in amounts of dietary starch, in total calories, or in the timing of feedings, as long as long-term caloric intake remains low enough to permit control of adiposity. Between-meal snacks and bedtime feedings are usually not necessary or desirable in obese diabetics.

On the other hand, lean patients with no endogenous insulin require approaches that are quite different in several respects. Calories should not be restricted below normal levels. It is usually desirable to provide smaller meals and one to three between-meal snacks in order to distribute food consumption to match roughly the time-action pattern of administered insulin. This mitigates postprandial surges of hyperglycemia and protects against hypoglycemia. In contrast to the typical situation in obese diabetics, no endogenous insulin is available in response to meals. In these lean, insulin-dependent patients it is usually desirable to provide for a considerable degree of consistency and predictability of the distribution, amounts, and characteristics of the feedings. In contrast to the therapy of obese diabetics, these patients also need considerable instruction on how to adjust the diet to contend with vagaries that may include unavoidable delay of meals, unusual exercise, complicating illnesses, management and prevention of hypoglycemic episodes, etc.

In both of the major types of diabetes there is decreasing emphasis on the priority of carbohydrate restriction.^{21,22} There are two reasons for this. First, it is now clear that insulin requirement is, in the long run,

more related to total fuel supply than to the amount of dietary carbohydrate itself. With excellent help from other elements of the body economy, the liver can make glucose readily out of a variety of sources. It is now evident that diets generous in starch are very well tolerated by both insulin-dependent and insulin-independent diabetics, provided that levels of calorie consumption are appropriate. Both plasma glucose and lipid levels usually respond quite favorably to such regimens. Even diabetics with Type IV hyperlipoproteinemia do well on liberal starch diets when levels of dietary sugar and calories are appropriately controlled.

Second, if levels of carbohydrate are sharply restricted, it is difficult to construct diets that are not high in fat and cholesterol. In the United States traditional diabetic diets of the past contained about 42 percent of calories as fat and generous amounts of cholesterol; while in Asian diabetics fat provided typically only about 15 percent of calories and levels of dietary cholesterol have been quite low. North American and Western European rates of coronary disease are exceedingly high in diabetics and coronary disease accounts for a majority of the deaths. In contrast, coronary atherosclerosis is far less common in the diabetics who consumed high starch, low fat, low cholesterol diets. These considerations have now led most Western diabetologists to prescribe diabetic diets that are lower in saturated fat and cholesterol than the previous traditional regimens. The calories derived from saturated fat can then be replaced if necessary by calories from three other sources: starch, vegetable fat, and protein. A typical "modernized" diabetic diet for a person in a Western society has the following characteristics: It contains a number of calories adequate to reach or maintain optimum weight. Refined sugars are proscribed or sharply limited, but about 10 to 15 percent of calories consist of sugars from natural sources such as fruit, vegetables, and milk. Fat is limited to about 25 to 35 percent of calories. Usually it is possible to reduce ani-

mal fat and cholesterol considerably without making the regimen unattractive or unfeasible. Saturated fat makes up only about 10 to 15 percent of calories in such a regimen (about half as much as the typical American diet), while vegetable fat (mono- and polyunsaturates) supplies about 15 to 20 percent of calories. In most adult patients, levels of protein are not critical and may range from as low as 12 percent of calories to as high as 24 percent depending on food preferences, food budgets, and other factors. Children, and women who are pregnant or lactating, require at least 1.5 g per kilogram. The remainder of calories, usually 30 to 40 percent, are derived from complex carbohydrates (mainly starches).

The most difficult aspects of the implementation of these "modernized diabetic diets" has been persuading dietitians, physicians, and patients that liberal amounts of starch are not bad for diabetes. In many instances these regimens do require the patient to eat even more starch than his family or friends. A review of the considerations discussed above, however, usually leads to an understanding and acceptance of this approach. A considerable body of recent evidence also supports the strategy of encouraging the ingestion of starches high in fiber.^{3,16}

Other recent publications have discussed the problems responsible for low rates of adherence to dietary prescriptions;²² methods for formulating diet prescriptions;²²⁻²⁵ the mechanics of developing and implementing prescriptions;²³⁻²⁵ the importance and methods of educating the patient;²³⁻²⁴ and the limited role of special foods such as artificial sweeteners, low-calorie and low-carbohydrate "diet" drinks and food products, fructose, sorbitol, alcohol, etc.²⁴ The texts edited by Marble et al. (Joslin's book)²⁶ and by Sussman and Metz²⁷ are good sources of information on diabetes. There is considerable evidence that only a minority of diabetics receive and follow on a long-term basis appropriate diet prescriptions.²² It is also clear that diet therapy is feasible and effective.^{22,28-32} □

1. *Diabetes Mellitus in Asia, 1970.* S. Tsuji and M. Wada, Editors. Excerpta Medica, Amsterdam, 1971
2. A. M. Cohen, A. Teitelbaum, and R. Saliternik, *Metabolism* 21: 235-240, 1972
3. T. L. Cleave in *The Saccharine Disease*. John Wright & Sons, Ltd., Bristol, 1974
4. K. M. West and J. M. Kalbfleisch, *Diabetes* 19: 656-663, 1970
5. K. M. West and J. M. Kalbfleisch, *Diabetes* 20: 99-108, 1971
6. K. M. West in *Is the Risk of Becoming Diabetic Affected by Sugar Consumption?* S. S. Hillebrand, Editor, pp. 33-43. International Sugar Research Foundation, Bethesda, 1974
7. K. M. West, *Diabetes* 23: 841-855, 1974
8. H. P. Himsworth, *Clin. Sci.* 2: 117-148, 1935-1936
9. *Is the Risk of Becoming Diabetic Affected by Sugar Consumption?* S. S. Hillebrand, Editor. International Sugar Research Foundation, Bethesda, 1974
10. H. P. Himsworth and E. M. Marshall, *Clin. Sci.* 2: 95-115, 1935
11. J. Booyens, M. de V. Frank, V. M. de Waal, G. D. Campbell, and M. D. Goldberg, *S. Afr. Med. J.* 44: 271-278, 1970
12. J. D. Baird, *Acta Diabetol. Lat.* (Suppl.) 9: 405-428, 1972
13. H. A. Kahn, J. B. Herman, J. H. Medalie, H. N. Neufeld, E. Riss, and U. Goldbourt, *J. Chron. Dis.* 23: 617-629, 1971
14. K. M. West in *The Regulation of the Adipose Tissue Mass*. J. Vague and J. Boyer, Editors. Excerpta Medica, Amsterdam, 1973
15. H. Keen in *Is the Risk of Becoming Diabetic Affected by Sugar Consumption?* S. S. Hillebrand, Editor, pp. 14-27. International Sugar Research Foundation, Bethesda, 1974
16. H. Trowell, *Lancet* II: 998-1002, 1974
17. K. Petersen, U. Schmitthenner, and L. Kerp, *Diabetologia* 10: 383, 1974
18. G. C. Gerritsen and W. E. Dulin, *Diabetologia* 10: 559-565, 1974
19. K. M. West, *Acta Diabetol. Lat.* (Suppl.) 9: 405-428, 1972
20. E. Miki and H. Maruyama in *Diabetes Mellitus in Asia 1970.* S. Tsuji and M. Wada, Editors. Excerpta Medica, Amsterdam, 1971
21. E. L. Bierman, M. J. Albrink, R. A. Arky, W. E. Connor, S. Dayton, N. Spritz, and D. Steinberg, *Diabetes* 20: 633-634, 1971
22. K. M. West, *Ann. Int. Med.* 79: 425-434, 1973

23. J. K. Davidson in *Current Therapy 1974*. H. F. Conn, Editor, pp. 386-408. W. B. Saunders Co., Philadelphia, 1974
24. K. M. West in *Nutritional Support of Medical Practice*. C. E. Anderson, D. B. Coursin, and H. A. Schneider, Editors. Harper-Row, New York, 1975
25. *Experimental and Therapeutic Dietetics* by M. A. Ohlson. Second edition. Burgess Publishing Co., Minneapolis, 1972
26. *Joslin's Diabetes Mellitus*. A. Marble, Editor. Eleventh edition. Lea & Febiger, Philadelphia, 1971
27. *Diabetes Mellitus: Diagnosis and Treatment*. K. Sussman and R. Metz, Editors, vol. IV. American Diabetes Association, New York, 1975 (in press)
28. R. L. Weinsier, A. Seeman, M. G. Herrera, J. J. Simmons, and M. E. Collins, *Diabetes* 23: 669-673, 1974
29. J. K. Davidson in *Epidemiologic Studies and Clinical Trials in Chronic Diseases*. Pp. 44-48. Pan-American Health Organization Proceedings of Symposium, Washington, D.C., 1972
30. G. W. Chance, E. C. Albutt, and S. M. Edkins, *Brit. Med. J.* 3: 616-618, 1969
31. D. B. Stone, *Am. J. Med. Sci.* 241: 64-70, 1961
32. D. B. Stone and W. E. Conner, *Diabetes* 12: 127-132, 1963

Nutrition Reviews

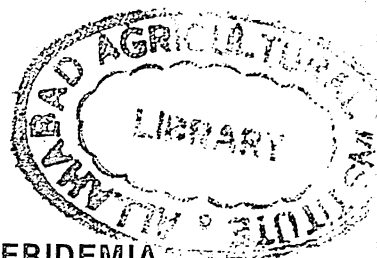
authoritative articles by distinguished international scientists • competent, critical, and knowledgeable reviews • interpretative summaries of important original clinical and experimental research • selected excerpts from classic publications marking milestones in nutrition research • informative special reports on government and organization actions • contributing editors review journals of widespread interest covering biochemistry, food science, public health, toxicology, dentistry, pediatrics, internal medicine, gastroenterology and other clinical specialities •

Send subscription and renewal requests to:

THE NUTRITION FOUNDATION
489 Fifth Avenue
New York, New York 10017

\$12.00 per year

\$6.00 for students



THE ROLE OF INSULIN IN HYPERTRIGLYCERIDEMIA

The authors postulate that cellular resistance to insulin may be the primary cause of endogenous hypertriglyceridemia. The correlations observed between glucose tolerance, insulin response, triglyceride production and plasma triglycerides seem to bear out this hypothesis.

Key Words: hypertriglyceridemia, VLDL-triglyceride, insulin resistance

Ever since the emergence of atherosclerosis and diabetes mellitus as major health problems, their association with altered carbohydrate and lipid metabolism has become clearly established. Much controversy, however, surrounds the pattern which these alterations follow.^{1,2} Whether the hyperinsulinemia observed in many patients with endogenous hypertriglyceridemia is a cause or a consequence has remained a debatable question.²

Olefsky and his co-workers³ postulate that central to the development of hypertriglyceridemia is the cellular resistance to insulin. This leads to a compensatory increase in plasma insulin levels which in turn acts on the liver to increase triglyceride synthesis. Consequently triglyceride concentration in the blood rises.

The above hypothesis was tested by these workers through a series of neatly planned experiments, carried out on 34 nonobese subjects having endogenous triglyceridemia or chemical diabetes. The subjects were placed on a liquid formula diet providing 42 percent fat and 43 percent carbohydrate. They were given the diet in four equal meals at three hour intervals, beginning at 8 AM.

Glucose and insulin response to the formula was tested hourly over a three-hour period, immediately following the 11 AM feeding. The results were expressed as the integrated area under the response curve. This was considered to represent the day long response of insulin and glucose to

food. Responses were also measured during a three hour glucose tolerance test, performed after the oral administration of 40 g of glucose per m².

Cellular insulin resistance was assessed by an ingenious technique. The release of endogenous insulin was suppressed by an intravenous infusion of epinephrine and propranolol and insulin and glucose were simultaneously infused. Under these circumstances, when a steady-state condition is reached, the blood glucose level is considered to be a measure of the response of the tissues to the steady-state insulin level. A high degree of correlation was observed between the integrated insulin response to the formula and the steady-state glucose concentration and between the former and plasma triglyceride levels. These observations were interpreted as indicating a decreased glucose tolerance with an increase in insulin levels and a concomitant increase in plasma triglyceride levels.

The VLDL-triglyceride production rate was studied by endogenous labeling with ³H-glycerol and studying the time-curve over the next 12 hours. From the values obtained for plasma VLDL-triglycerides and for plasma volume, the turnover rate of this lipoprotein fraction was calculated. The experiment being done under steady-state conditions, the turnover rate is expected to equal the production rate. This parameter was also found to correlate highly with the insulin response as well as with the plasma triglyceride concentration. The latter implied that the increased hepatic triglyceride synthesis may lead to the increase in plasma triglycerides.

The observations made in the study have been interpreted as indicating that a primary cellular resistance to insulin underlies the development of endogenous triglyceridemia. This interpretation is based solely on the correlations obtained between the various parameters. Correlations, however, do not necessarily imply a causal relationship; nor should the corroboration of a hypothesis be considered as proving its veracity. The interesting concepts presented by the authors, however, provide a basis for further study of the pathogenesis of hyperlipemias. Lately, it has been postulated that decreased levels of glucagon may be responsible for the development of endogenous triglyceridemia.⁴ It had been suggested earlier that the effective ratio of insulin to glucagon may be important in the pathogenesis of diabetes mellitus.⁵ A similar bihormonal

control may also regulate lipid metabolism. □

1. E. A. Nikkila: Control of Plasma and Liver Triglyceride Kinetics by Carbohydrate Metabolism and Insulin. *Adv. Lipid Res.* 7: 63-134, 1969
2. R. J. Havel: Pathogenesis, Differentiation and Management of Hypertriglyceridemia. *Adv. Int. Med.* 15: 117-154, 1969
3. J. M. Olefsky, J. W. Farquhar and G. M. Reaven: Reappraisal of the Role of Insulin in Hypertriglyceridemia. *Am. J. Med.* 57: 551-560, 1974
4. R. P. Eaton, D. S. Schade and M. Conway: Decreased Glucagon Activity: A Mechanism for Genetic and Acquired Hyperlipaemia. *Lancet II*: 1545-1547, 1974
5. R. H. Unger: Glucagon Physiology and Pathophysiology. *New Engl. J. Med.* 285: 443-449, 1971

HISTIDINE: AN ESSENTIAL AMINO ACID FOR NORMAL ADULTS

Ingestion of a histidine-deficient diet was accompanied by a decreased N balance, the development of anemia and a marked decrease in serum and muscle histidine content. These were all alleviated by the addition of histidine to the diet.

Key Words: histidine, N balance, anemia, humans

Although histidine has been firmly established as a dietary essential amino acid for growing animals of several species, its necessity in the diet of normal adults has not been clearly identified.¹ Based on recent reports that histidine is essential in the diet of chronically uremic patients, Kopple and Swensid re-investigated this problem. They compared the metabolic response to deletion of histidine from the diet of normal and of chronically uremic adult males.²

The subjects were four adult men with no evidence of renal disease and three men with advanced chronic renal failure. They lived in a metabolic unit and were fed first for several weeks a diet of ordinary foods containing 40 g protein, most of it from

eggs or beef. For the next 35 ± 2 days, they received a purified diet in which about 92 percent of the N was supplied as L-amino acids in the proportions present in egg except that only 60 mg histidine were provided daily. In the final period of 31 ± 5 days a diet isonitrogenous to that in period two was provided, containing 1210 mg histidine. During this final period one uremic subject received 590 mg histidine for four days, left the ward for three days and then received the original ordinary diet for 11 days. In the amino acid diets energy was mainly supplied by wheat-starch bread and vegetable fat. Supplemental vitamins and minerals were provided although the magnesium intake was only 50 percent of the recommended allowance.

Nitrogen balances were conducted throughout the study in consecutive

periods, usually of five days duration, using well-established techniques. Balances were adjusted for changes in body urea content but not for losses from skin, respiration or blood sampling. Fasting blood samples were drawn several times weekly. At the end of each period a biopsy sample of gastrocnemius muscle was also taken.

The initial N balances were generally negative, perhaps reflecting a larger N intake prior to the study but by the end of the first period the balances were even or positive in six of the seven subjects. The change to a histidine-deficient diet tended to reduce the N balance and by the end of this period balances were negative in all subjects. Addition of histidine in period three produced positive balances in five of the six subjects who completed this period successfully. Data obtained during the last seven to 12 days on each diet averaged $+ 0.65 \pm .59$ (SD), $-.48 \pm .44$ and $+ .70 \pm 1.2$ g per day for the natural, histidine-deficient and histidine-supplemented diets. The differences between period two and either period one or three were highly significant statistically. Serum albumin and body weights did not change remarkably during the course of the study.

Within 23 hours of initiation of the histidine-deficient period plasma histidine fell by 52 percent. By the end of this period it was only 17 percent of the control values. Recovery of plasma histidine was slower and more erratic during the repletion period; at the end of this period five of the seven subjects had regained their normal levels. Muscle histidine levels behaved in a similar fashion although the decrease at the end of depletion was only to 38 percent of normal. The hematocrits were lower in uremic than in normal subjects but in all subjects the values were decreased by 25 percent during the depletion period. Repletion caused an increase after a lag period of two to ten days. In five subjects their hematocrits were still rising at the end of the repletion period, although normal values were not restored. Reticulocyte counts were not different in periods one and two but increased during repletion from values of 1.8 percent to 5.2

percent. Serum iron values rose from about 85 to 148 μg per 100 ml during the histidine-depletion period. They fell during repletion to 57 μg within 48 hours, a level which was maintained to the end of the experiment. These differences were highly significant. Iron-binding capacity of the serum decreased 17 percent during period one, did not change further during depletion but rose again to control values during repletion.

Clinical symptoms developed gradually during depletion and included a sense of fatigue and a lack of energy. Poor appetite and nausea were frequent. Irritability, hostility, anxiety and confusion were also noted. Five subjects developed a dry, scaly dermatitis. Upon starting the repletion diet these symptoms disappeared but only gradually. Despite the improvement in all symptoms, complete recovery was not reported until the subjects returned to a normal diet.

These findings of negative N balance, markedly decreased serum and muscle histidine, anemia and clinical symptoms and their reversal by adding only histidine to the diet suggest that histidine is an essential amino acid for both normal and chronically uremic adults. The difference between these findings and those of other investigators^{3,4} may be ascribed to the use in this experiment of a longer depletion period and a lower histidine intake during depletion.

The slow development of negative N balance during depletion could have been a reflection of utilization of the 60 mg of histidine present in the depletion diet. It could have also reflected a re-utilization of histidine released from catabolism of histidine-rich proteins such as hemoglobin or the histidine derivative, carnosine.

Of the clinical symptoms described, the skin lesions and severe anemia are peculiar to histidine deficiency. They point to the desirability of further quantitating the histidine requirement and to study of the mechanisms leading to these abnormalities. The possible importance of histidine in other anemias may also be worthy of consideration. □

1. M. I. Irwin and D. M. Hegsted: A Conspectus of Research on Amino Acid Requirements of Man. *J. Nutrition* 101: 539-566, 1971
2. J. D. Kopple and M. E. Swendseid: Evidence that Histidine is an Essential Amino Acid in Normal and Chronically Uremic Man. *J. Clin. Invest.* 55: 881-891, 1975
3. W. C. Rose, W. J. Haines and D. T. Warner: The Amino Acid Requirements of Man. III. The Role of Isoleucine: Additional Evidence Concerning Histidine. *J. Biol. Chem.* 193: 605-612, 1951
4. A. A. Albanese, L. E. Holt, Jr., J. E. Frankston and V. Irby: Observations on a Histidine Deficient Diet in Man. *Bull. Johns Hopkins Hosp.* 74: 251-258, 1944

THE SECRETION OF BILE

A sensitive radioimmunoassay for conjugated bile salts has been used to study their enterohepatic circulation in health and disease. The subcellular site of action of taurocholate on biliary lipid synthesis and secretion has been clarified.

Key Words: enterohepatic circulation, bile acids, biliary lipid, cholecystectomy, ileal resection

Bile salts, of which the most important examples are the salts of glycocholic and taurocholic acid, undergo an enterohepatic circulation. This involves being synthesized from cholesterol in the liver, then secreted via the bile canaliculi and stored in the gall bladder from which phasic ejection takes place into the small bowel in response to meals. The salts, beside facilitating the absorption of dietary lipid, enhance the further synthesis and secretion of bile by an action on the hepatocyte. The bile salts are absorbed in the small intestine and extracted from the portal circulation by the liver. When the hepatic influx of bile salts is reduced there is an associated decrease in the secretion of biliary lipids which is more pronounced for phospholipids than cholesterol. This results in a bile with a relatively high cholesterol concentration which may be conducive to the formation of gallstones.¹ Increasing the hepatic uptake of bile salts by their oral administration for example, leads conversely to increased bile production in which the concentration of cholesterol is reduced relative to that of bile salts.² It is apparent that the circulation of bile salts is of great importance, intrinsically and because of the effects it has upon the synthesis and secretion of biliary lipid. Two pieces of recent

work made significant contributions to our understanding of these processes.

Using a sensitive radioimmunoassay which recognizes conjugates of cholic acid (CCA),³ LaRusso and his colleagues studied changes in serum CCA occurring during a 24-hour period in normal volunteers and patients who had undergone cholecystectomy or ileal resection.⁴ Each subject had an indwelling venous cannula and was allowed to move about the metabolic unit during the study period. During this time three identical liquid meals were given at 0800, 1230 and 1730 hours. The meal provided 30 kcal per kilogram of body weight and was 40 percent protein, 40 percent carbohydrate and 20 percent fat. Blood samples were taken every 15 to 30 minutes during the day and at one to two hour intervals throughout the night.

In eight normal volunteers serum CCA levels rose to a peak 90 to 120 minutes after each test meal and returned to preprandial levels within four hours. The rise in CCA concentration was three- to ten-fold and did not change significantly between meals. The causal relationship between ingestion of food and changes in serum CCA was confirmed by having four of the subjects take only breakfast on another day. After the characteristic rise and fall of CCA, the levels remained low

for the remainder of the 24-hour study period. In five patients who had had a cholecystectomy for cholelithiasis there was a rise in serum CCA to a maximum 75 to 105 minutes after the first meal followed by a failure to return to basal levels. The mean (\pm S.E.) degree of rise was smaller than in the normal volunteers being from 0.33 ± 0.05 to 0.79 ± 0.07 μ moles per liter compared with 0.42 ± 0.07 to 1.58 ± 0.17 μ moles per liter. When the integrated area under the 24 hour curve, taken as a reflection of the total CCA secreted, was compared no significant difference was noted. Six patients who had had ileal resection for regional ileitis or in one case endometriosis, were also studied. They showed a small rise from 0.20 ± 0.01 to 0.50 ± 0.06 μ moles per liter after the first meal, a smaller rise after the midday meal and no CCA response to the third meal. The integrated area under their 24 hour CCA curve was one-third that of the normal and cholecystectomy subjects. The patterns of CCA curves in individuals were not related to the amount of ileum resected or the degree of steatorrhea.

Peripheral serum bile acid concentration must reflect the net balance between hepatic output and uptake. The detection of measurable levels during fasting indicates continuing bile salt secretion into the intestine. The postprandial rise, which also occurs after ingestion of solid meals, is a measure of increased biliary secretion and gall bladder contraction. In normal individuals the return to basal levels in the postprandial period indicates hepatic clearance of serum bile salts and storage by the gall bladder. In postcholecystectomy patients this latter mechanism cannot occur and so increased secretion into the gut continues throughout the day resulting in a high plateau of serum levels. In contrast, the subjects who had ileal resection are unable to absorb bile salts efficiently and lose them in the stools. Consequently the serum peaks are smaller. The authors suggest that the diminishing responses to the second and third meals are due to hepatic inability to maintain an adequate rate of

bile acid synthesis to balance the losses in the stools.

It is apparent that the radioimmunoassay for CCA is a powerful tool to study biliary physiology and pathology in man. The sensitivity and precision of the method make it feasible to design ethically acceptable tests which will eventually have a diagnostic clinical value as well as being useful for research.

Another aspect of biliary function was studied by Gregory and his colleagues.⁵ Using an isolated perfused rat liver model, they investigated the role of bile salts in stimulating the synthesis and secretion of biliary lipids. The patterns of cholesterol, lecithin and bile salt secretion occurring in normal and disease states support the concept of a homeostatic relationship between bile acid fluxes and biliary lipid secretion, but the exact site of action of the bile salts remains undefined. Biliary lecithins are known to be synthesized in the liver via the CDP-choline diglyceride pathway.⁶ They are also rich in palmitic and linoleic acids but transacylation must also occur as one species is rich in arachidonic acid.⁷ Evidence indicates that the microsomal fraction contains the sole hepatic subcellular membranes with CDP choline transferase activity necessary for lecithin synthesis *ab initio*. The role of the biliary canalicular membranes in lecithin synthesis and transport has not yet been investigated.

Gregory et al. perfused isolated rat livers with a red blood cell enriched, physiological medium in a closed system to which sodium taurocholate was added in some experiments. Radioactive palmitic acid, linoleic acid, phosphate or mevalonic acid were infused. Bile was collected at intervals before and afterwards. At the end of the experiment the livers were weighed, placed in a chilled buffer and homogenized. Bile canalicular membranes and microsomes were prepared by ultracentrifugation. The purity of the membrane fractions was confirmed by enzyme analysis and electron microscopy. The microsomes were rich in glucose-6-phosphatase and the canalicular membranes in 5'-nucleotidase and Mg^{++} -

dependent ATPase. Each fraction prepared by centrifugation showed less than 10 percent contamination. CDP-choline diglyceride transferase activity was present in the microsomes but not in the canaliculi. Electron microscopy showed the bile canalicular fraction to contain characteristic tight junctions and fragmented membranes.

Under control conditions the secretion of biliary lipid by the liver was very low (phospholipid 0.2 and cholesterol 0.05 μ moles per hour). The addition of taurocholate, however, caused an immediate ten- and four-fold increase respectively in the secretion rates. The incorporation of palmitic and linoleic acids into lecithin occurred rapidly. The highest specific activity of the microsomal fraction occurred 30 minutes after the infusion of radioactive precursors at which time its specific activity was greater than that of the canalicular fraction. During the next study period, from 30 to 60 minutes, the specific activity of biliary lecithin was greater than either the microsomal or canalicular fraction. This difference became greater during the last study period from 60 to 90 minutes.

Choline was incorporated into linoleyl lecithin in both membrane fractions and bile more rapidly than into arachidonyl lecithin as was phosphate but the pattern of phosphate incorporation differed from that of choline or fatty acid. At 30 minutes after injection the lecithin phosphate specific activity was greatest in the biliary canalicular membranes, intermediate in bile and lowest in the microsomal fraction. Furthermore phosphate incorporation to linoleyl and arachidonyl lecithins occurred equally. By 60 to 90 minutes the biliary lecithin phosphate activity was highest.

The addition of taurocholate to the perfusion medium markedly stimulated the incorporation of choline and phosphate into lecithin. The incorporation curves of mevalonic acid into cholesterol in the microsomes and canaliculi were almost identical indicating a rapid intracellular transfer of this molecule in the presence of taurocholate.

This work elegantly demonstrated that bile canalicular membranes do not synthesize biliary lecithin, that both linoleyl and arachidonyl lecithins originate in the hepatic microsomes and that lecithin is transported rapidly from its site of synthesis to the point of secretion. The authors interpret the phosphate incorporation data as possibly due to incomplete equilibration of the inorganic radioactive phosphate with a large ATP pool occurring in the early part of the study. Another possibility is that the observed pattern of phosphate incorporation is due to phosphate being involved in secretion of lecithin as well as in its synthesis. The early incorporation of phosphate in the canaliculi is due to the secretion of pre-formed lecithin. Taurocholate is clearly essential for both the synthesis and secretion of biliary lipid and may act specifically on the CDP-choline pathway.^{2,6} The existence of a transport protein or proteins for both lipids and bile salts was considered by the authors as a possible mechanism for intrahepatocyte transport and site of action for stimulation by bile acids. This was not included in their study. Proteins have been isolated from hepatocyte cell sap which bind both bile acids⁸ and biliary lipids.⁹ Further work is necessary to clarify the relationships of these binding proteins to bile salt and lipid metabolism and secretion. □

1. W. H. Admirand and D. M. Small: The Physicochemical Basis of Cholesterol Gallstone Formation in Man. *J. Clin. Invest.* 47: 1043-1052, 1968
2. J. A. Balint, D. A. Beeler, E. C. Kyriakides and D. H. Treble: The Effect of Bile Salt upon Lecithin Synthesis. *J. Lab. Clin. Med.* 77: 122-133, 1971
3. W. J. Simmonds, M. G. Korman, V. L. W. Go and A. F. Hofmann: Radioimmunoassay of Conjugated Cholyl Bile Acids in Serum. *Gastroenterology* 65: 705-711, 1973
4. N. F. LaRusso, M. G. Korman, N. E. Hoffman and A. F. Hofmann: Dynamics of the Enterohepatic Circulation of Bile Acids. Postprandial Serum Concentrations of Conjugates of Cholic Acid in Healthy, Cholecystectomized Patients,

- and Patients with Bile Acid Malabsorption. *New Engl. J. Med.* 291: 689-692, 1974
5. D. H. Gregory, Z. R. Vlachcevic, P. Schatzki and L. Swell: Mechanism of Secretion of Biliary Lipids. I. Role of Bile Canalicular and Microsomal Membranes in the Synthesis and Transport of Biliary Lecithin and Cholesterol. *J. Clin. Invest.* 55: 105-114, 1975
 6. J. A. Balint, D. A. Beeler, D. H. Treble and H. L. Spitzer: Studies in the Biosynthesis of Hepatic and Biliary Lecithins. *J. Lipid Res.* 8: 486-493, 1967
 7. R. Sundler, G. Arvidson and B. Akesson: Pathways for the Incorporation of Choline into Rat Liver Phosphatidylcholines In Vivo. *Biochim. Biophys. Acta* 280: 559-568, 1972
 8. R. F. Hanson, K. McCoy and M. E. Dempsey: The Role of a Carrier Protein in Bile Acid Biosynthesis. *Gastroenterology* 64: 154, 1973
 9. T. J. Scallen, M. W. Schuster and A. K. Dhar: Evidence for a Noncatalytic Carrier Protein in Cholesterol Biosynthesis. *J. Biol. Chem.* 246: 224-230, 1971

PHARYNGEAL LIPASE ACTIVITY IN MAN

Lipolytic activity was found in esophageal aspirates of human subjects and was not present in saliva.

Key Words: gastric lipolysis, humans

In the classical concept of lipid digestion in animals with simple stomachs, it is stated that enzymic lipolysis is initiated by pancreatic lipase acting in the duodenum. Limited lipolysis is recognized to occur in the stomach, generally attributed to action of a weak gastric lipase, primarily affecting dietary lipid emulsions. The source of gastric lipase has not been identified except in the calf¹ in which lipolytic activity was demonstrated in glandular tissue of the tongue, pharynx and upper esophagus. Recently a potent lipase was demonstrated in serous glands of the rat tongue.² Effective gastric hydrolysis of dietary triglycerides to partial glycerides and free fatty acids was catalyzed by lingual lipase.

Evidence, presented by Hamosh et al.,³ demonstrates that esophageal aspirates from man contain lipolytic activity closely resembling that found in the tongue and in the stomach of rats.

The subjects were 11 female and six male volunteers, between the ages of 20 and 54 years. They ate a regular diet until fasted overnight prior to the tests. Esophageal and gastric aspirates were obtained with a nasogastric tube without anesthesia. Samples were obtained at two levels of the esophagus and from the

fundus of the stomach. They were taken while the subject drank 15 to 30 ml of a cream-milk mixture followed by 15 to 45 ml of water. Esophageal samples were saved for analysis only if they were water clear. Gastric samples, taken last, usually contained a small amount of triglyceride from the milk-cream mixture. Salivary gland secretions were collected simultaneously with devices placed directly over the gland orifices.

Lipolytic activity was assayed by the amount of chylomicron triglyceride hydrolyzed to diglyceride, monoglyceride, glycerol and FFA. The assay mixture contained doubly labeled chylomicrons, sodium citrate-phosphate buffer, bovine serum albumin and the sample of aspirate. The chylomicrons were isolated by centrifugation of thoracic duct chyle from fasted rats tube-fed corn oil containing 1-¹⁴C palmitic acid and trioleoyl(2-³H) glycerol and suspended in 4 percent albumin solution.

After incubation at 38°C for 15 to 60 minutes the lipids were extracted with hexane, separated by thin layer chromatography and counted for ¹⁴C and ³H content. In some cases nonradioactive lipids were used. In these cases, FFA in the hexane extract were determined by titration. The glycerides were separated by

column chromatography and determined photometrically.

Aspirates from both esophagus and stomach contained lipolytic activity that was maximal at pH 5.4 and hydrolyzed chylomicron triglyceride, producing in mole percent of the glyceryl products 67 to 78 diglycerides, 21 to 26 monoglycerides and 8 glycerol. The rate of chylomicron triglyceride hydrolyzed varied from none to 357 nmoles per minute at pH 5.4 and was very slight at pH 7.4. Salivary secretions contained essentially no lipolytic activity.

In further tests the hydrolysis of milk and corn oil triglycerides in the stomach were examined. The overnight fasted subjects were given 30 or 60 ml of liquid test meals containing 11 to 13 percent triglyceride, either as a cream-milk mixture or as a corn oil-water mixture. Gastric samples were obtained before and at various periods up to ten minutes after consuming these meals. Corn oil triglyceride was hydrolyzed as rapidly as milk triglyceride. Three to 12 percent of the triglyceride was hydrolyzed within four minutes. There was no further hydrolysis up to ten minutes. Most of the glyceryl products were diglyceride, with monoglyceride and FFA being minor products.

In a final, *in vitro* test, it was shown that addition of products of triglyceride hydrolysis to triolein in 1 percent gelatin solution would stimulate emulsification of the triolein upon shaking at pH 5.4. This indicated that these hydrolytic products, added in amounts and proportion similar to those found in the stomach contents, could facilitate emulsification of triglyceride in the stomach.

These data demonstrate that enzymic lipolysis in man is initiated by secretions entering the esophagus at the pharyngeal region and not by saliva. The di- and monoglycerides and FFA produced may stimulate emulsification of triglycerides, thus increasing the rate of lipolysis in the stomach. □

1. H. A. Ramsey, G. H. Wise and S. B. Tove: Esterolytic Activity of Certain Alimentary and Related Tissues from Cattle in Different Age Groups. *J. Dairy Sci.* 39: 1312-1322, 1956
2. M. Hamosh and R. O. Scow: Lingual Lipase and Its Role in the Digestion of Dietary Fat. *J. Clin. Invest.* 52: 88-95, 1973
3. M. Hamosh, H. L. Klaeveman, R. O. Wolf and R. O. Scow: Pharyngeal Lipase and Digestion of Dietary Triglyceride in Man. *J. Clin. Invest.* 55: 908-913, 1975

VITAMIN E THERAPY IN PREMATURE BABIES

The pathological basis for the hemolytic anemia of vitamin E deficiency is discussed. New work comparing the use of two different therapeutic forms of vitamin E in premature babies is evaluated.

Key Words: vitamin E deficiency, hemolytic anemia, iron therapy, premature babies

Vitamin E deficiency in older children and adults is very rare because this vitamin is widespread in plants and because vitamin E stores in the body are considerable. Vitamin E is present in the membrane fraction of red cells¹ and plays an important role in the function of cellular membranes, not only the outer cell membrane, but also those of intracellular organelles such as the

mitochondria. Recent variations have been proposed on the classical Danielli-Davson hypothesis that the cell membrane is a bilayer construction of phospholipid and protein. Nevertheless, the importance of phospholipid and its maintenance in an appropriate form is an essential assumption of all theories. It has been suggested that vitamin E acts as an antioxidant preventing the formation of peroxides,² particularly preventing peroxidation of membrane

lipids.³ Alternatively by complexing with phospholipids, vitamin E may control membrane stability.⁴

Vitamin E deficiency can be induced in experimental animals and is present on a pathological basis in individuals who have α - β -lipoproteinemia. This is a rare hereditary disorder, with an autosomal recessive form of inheritance where the homozygote lacks β -lipoprotein and vitamin E deficiency thus ensues.

In this condition acanthrocytosis is present, i.e. the red cells are spiny with numerous projections from their surface. In such individuals and in the vitamin E-deficient duckling, direct pathology of cell mitochondria and other organelle membranes has been found by ultrastructural studies with the electron microscope.⁵ Instead of clearly defined, closely apposed double membranes as in the normal situation, there is in the membranes a general fuzziness and lack of definition. In the α - β -lipoproteinemic children, as well as in the ducklings, several months of therapy with vitamin E are necessary to restore the normal picture.

The population most at risk in the development of vitamin E deficiency is the premature baby. These infants have low stores at birth, and their capacity to absorb this vitamin is diminished compared with more mature infants.⁶ Since hemopoietic tissue is among those with the most rapid turnover, it is not surprising that vitamin E deficiency manifests itself mainly as a hemolytic anemia with morphological evidence of membrane abnormality i.e. acanthrocytosis. In addition there is evidence that vitamin E has a stimulatory effect on the synthesis of heme,⁷ which may also be contributory to the anemia.

There are two other factors to be considered. First, the degree of saturation of the fats ingested affects the requirement for vitamin E. Thus the breast fed baby requires less of the vitamin than the bottle fed, since proprietary milks have a considerably higher amount of polyunsaturated fatty acids than breast milk. Small premature infants can absorb these

fatty acids well, but not the vitamin E to protect their red cells from hemolysis. The second factor thought to be contributory to vitamin E deficiency hemolysis is iron. This mineral can act as a co-factor in vitro, catalyzing the oxidative breakdown of red cell lipids. Melhorn and his co-workers⁸ showed that preterm babies given iron alone are significantly more anemic than those given iron and vitamin E, vitamin E alone or no supplement. Therefore it has been suggested that, since even the preterm infant will not exhaust his iron stores before two to three months of age, it would be better not to give therapeutic iron before this period elapsed.⁹

Vitamin E has been commonly administered as the fat-soluble form, α -tocopherol acetate (TA). A water-soluble form, α -tocopherol polyethylene glycol 1000 succinate (TPGS), is now available. The relative ease of absorption of this substance in preterm infants is the subject of a recent report by Gross and Melhorn.¹⁰ The effects of concurrently administered iron were also studied.

Infants of less than 36 weeks gestation and less than 2000 g birth weight were studied, those with anemia being excluded. In the first study, 30 infants were divided into three groups and fed either 25 IU of TPGS daily, the same amount of TA or no supplement at all. This last group served as a control. Those infants fed the water-soluble vitamin TPGS had a significantly smaller decline in hemoglobin over the ensuing six weeks as compared to the control infants, i.e. 22.5 and 34.0 percent respectively. Those infants fed the fat-soluble vitamin (TA) gave intermediate results with a 27.5 percent decline. Thus water-soluble vitamin E appears to be more efficacious in preventing hemolysis in the newborn. Absorption studies in three-day-old premature infants showed that TPGS was better absorbed than TA.

A further study was initiated to show the effects of iron. Forty-five infants were divided into three groups and given fat-soluble TA with 13.7 mg of elemental iron or with trace quantities of elemental iron.

A third group was given water-soluble TPGS with 15.7 mg of elemental iron per day. This last group was also unavoidably given 6.6 IU of TA since this amount was naturally present in the milk formula. Again, those infants receiving the water-soluble vitamin had the lowest fall in hemoglobin and those receiving the fat-soluble vitamin with an iron supplement had the larger fall. Thus iron appeared to be deleterious to the maintenance of hemoglobin levels. Both groups of infants given TPGS had higher serum tocopherol levels than did the other groups.

In four infants with low tocopherol levels the level of erythrocyte membrane phosphatidyl ethanolamine was low compared to that found in vitamin E-replete babies.

The underlying basis of the interference of iron with vitamin E is not clear. It appears to be partly due to interference with absorption since, of the two groups fed TPGS, those without additional iron had higher serum tocopherol levels than those with iron. Nevertheless the constitution of the formulas in these two groups was not exactly the same. As the authors point out, it would have been better to have included a comparable formula with trace quantities of iron in the study.

Thus the water-soluble vitamin E is to be preferred as therapy for small premature babies, probably because absorption of this vitamin is unaffected by deficiency of bile salt or pancreatic enzyme secretion. The role of iron in vitamin E metabolism requires further elucidation but for practical purposes the two should not be given to-

gether unless there is evidence of iron deficiency. □

1. R. Silber, R. Winter and H. J. Kayden: Tocopherol Transport in the Rat Erythrocyte. *J. Clin. Invest.* 48: 2089-2095, 1969
2. A. L. Tappel: Vitamin E as the Biological Lipid Antioxidant. *Vitamins Hormones* 20: 493-510, 1962
3. P. B. McCay, P. M. Pfeifer and W. H. Stipe: Vitamin E Protection of Membrane Lipids during Electron Transport Functions. *Ann. N.Y. Acad. Sci.* 203: 62-73, 1972
4. J. A. Lucy: Functional and Structural Aspects of Biological Membranes: A Suggested Structural Role for Vitamin E in the Control of Membrane Permeability and Stability. *Ann. N.Y. Acad. Sci.* 203: 4-11, 1972
5. I. Molenaar, C. E. Hulstaert, J. Vos and F. A. Hommes in *Therapeutic Aspects of Nutrition*. J. H. P. Jonxix, H. K. A. Visser and J. A. Troelstra, Editors, Nutricia Symposium, pp. 41-55. H. E. Stenfort Kroese, Leiden, 1973
6. D. K. Melhorn and S. Gross: Vitamin E-Dependent Anemia in the Premature Infant. II. Relationships between Gestational Age and Absorption of Vitamin E. *J. Pediat.* 79: 581-588, 1971
7. P. P. Nair: Vitamin E and Metabolic Regulation. *Ann. N. Y. Acad. Sci.* 203: 53-61, 1972
8. D. K. Melhorn, S. Gross and G. Childers: Vitamin E-Dependent Anemia in the Premature Infant. I. Effects of Large Doses of Medicinal Iron. *J. Pediat.* 79: 569-580, 1971
9. P. R. Dallman: Iron, Vitamin E, and Folate in the Preterm Infant. *J. Pediat.* 85: 742-752, 1974
10. S. Gross and D. K. Melhorn: Vitamin E-Dependent Anemia in the Premature Infant. *J. Pediat.* 85: 753-759, 1974

RECENT CLINICAL CORRELATES OF VITAMIN D METABOLITES AND CALCIUM METABOLISM

Several types of bone disease are related to abnormalities in the metabolism of vitamin D. The synthetic 1-OH hydroxycholecalciferol appears to have considerable potential as a therapeutic agent.

Key Words: 25-hydroxycholecalciferol (25-OH-HCC), calcium absorption

Vitamin D or cholecalciferol is formed by the action of ultraviolet light on 7-dehydrocholesterol in the skin and is available as a fat soluble sterol in the diet. While the major circulating form of vitamin D is 25-hydroxycholecalciferol (25-OH-HCC), the most potent form of vitamin D with regard to the intestinal transport of calcium is 1,25 hydroxycholecalciferol (1,25 OH-HCC). In recent years it has been shown that vitamin D is metabolized to 25-OH-HCC in the liver while the kidney is the major site of formation of 1,25 OH-HCC. The principal functions of vitamin D include a synergistic effect with parathyroid hormone in the mobilization of bone, and the intestinal absorption of dietary calcium. Vitamin D appears to be an essential factor in the synthesis of calcium-binding protein, the carrier for calcium transport across the intestinal mucosal cell. Since 1,25 OH-HCC is the most potent form of vitamin D and requires hepatic and renal metabolism for its formation, one might predict that diseases of these organs would affect the intestinal transport and metabolism of calcium. Several recent articles placed these physiologic observations in a clinical concept.

Oettinger and his co-workers studied the intestinal absorption of calcium in seven patients with endstage renal disease, before and after bilateral nephrectomy.¹ The patients were maintained on hemodialysis while awaiting renal transplant. Calcium absorption was measured using a double

isotope method whereby ^{45}Ca was administered orally and ^{57}Ca was administered intravenously. The ratio of the isotopes in the blood at 24 hours was used to calculate the intestinal absorption of calcium. Prior to nephrectomy the mean calcium absorption was 20.2 percent. Following bilateral nephrectomy calcium absorption decreased significantly to a mean of 12.7 percent. The decrease in calcium absorption was ascribed to an absence of renal 1-hydroxylation of vitamin D_3 , although levels of 1,25 OH-HCC were not measured directly.

In a recent investigation of the etiology of hypocalcemia and bone thinning in 56 epileptic children being maintained on anticonvulsant therapy, Hahn and his co-workers measured the effects of phenobarbital and diphenylhydantoin, singly or in combination, on levels of calcium, serum 25-OH-HCC and bone mass.² The patients were matched with a control group of the same age and sex in which the exposure to sunlight and to vitamin D (3500 I.U. per week) was similar. The incidence of hypocalcemia was 4 percent in the drug-treated group while serum calcium levels as a whole were significantly less than in the control group. There was a marked increase, however, in liver and bone alkaline phosphatase as well as a definite decrease in levels of serum 25-OH-HCC in the drug-treatment groups. Bone mass measured in the radius was significantly decreased in patients receiving the combined anticonvulsant therapy. There was a positive correlation of serum calcium and serum 25-OH-HCC in the drug-treated groups but not in the control group. Thus anticonvulsant therapy

with diphenylhydantoin and phenobarbital has a suppressant effect on levels of 25-OH-HCC with a resultant decrease in serum calcium levels and in bone mass. A physiologic basis for these clinical findings is the in vitro observation that liver microsomes from phenobarbital-treated animals show enhanced conversion of 25-OH-HCC to inactive polar products which are excreted in the bile.³ These data were correlated with clinical observations showing decreased plasma half-life of tritium-labeled vitamin D₃ with increased conversion to polar metabolites in patient volunteers receiving chronic phenobarbital therapy. Anticonvulsant therapy may result in osteomalacia through increased hepatic microsomal inactivation of 25-OH-HCC.

The clinical use of 1 OH-HCC was described in studies of patients with intestinal malabsorption who were hospitalized in Paris, France.⁴ It has previously been shown to be effective in renal patients⁵ and in the chick. The synthetic 1 OH-HCC is as effective as 1,25 OH-HCC in promoting intestinal calcium transport.⁵ In the clinical study, the intestinal absorption of calcium and phosphorus and osteoclastic activity in bone biopsies were measured in two adult patients with celiac sprue and in two patients with intestinal stasis (bacterial overgrowth) syndromes. 1 OH-HCC was administered intravenously at a dose of 2.5 or 5.0 µg per 24 hours for a ten day period. With either dose there was a decrease in the fecal excretion of dietary calcium and phosphorus as well as a fall in urinary excretion of phosphorus. This effect could

have resulted from decreased parathyroid hormone activity or a direct effect of 1 OH-HCC on the renal medulla. Simultaneously, in bone biopsies osteoclastic activity increased in three patients while the percent of calcified osteoid was increased in each case. Thus a synthetic vitamin D compound which incorporates the normal renal step of 1-hydroxylation of cholecalciferol may be highly effective as an antirachitic compound. When widely available, this compound may prove highly effective in the treatment of disabling osteomalacia in patients with intestinal malabsorption syndromes. □

1. C. W. Oettinger, R. Merrill, T. Blanton and W. Briggs: Reduced Calcium Absorption after Nephrectomy in Uremic Patients. *New Engl. J. Med.* 291: 458-460, 1974
2. T. J. Hahn, B. A. Hendin, C. R. Scharp, V. C. Boisseau and J. G. Haddad: Serum 25-Hydroxycalciferol Levels and Bone Mass in Children on Chronic Anticonvulsant Therapy. *New Engl. J. Med.* 292: 550-554, 1975
3. T. J. Hahn, S. J. Birge, C. R. Scharp and L. V. Avioli: Phenobarbital-Induced Alterations in Vitamin D Metabolism. *J. Clin. Invest.* 51: 741-748, 1972
4. P. Bordier, M. P. Pechet, R. Hesse, P. Marie and H. Rasmussen: Response of Adult Patients with Osteomalacia to Treatment with Crystalline 1 α-Hydroxy Vitamin D₃. *New Engl. J. Med.* 291: 866-871, 1974
5. T. M. Chalmers, J. O. Hunter, M. W. Davie, K. F. Szoz, B. Pelc and E. Kodicek: 1-Alpha-Hydroxycholecalciferol as a Substitute for the Kidney Hormone 1,25-Dihydroxycholecalciferol in Chronic Renal Failure. *Lancet* II: 696-699, 1973

NUTRITION CLASSICS

THE
BIOCHEMICAL
JOURNAL

28 NOV 1973

VOLUME XXVIII: 916-925, 1934

CXXXI. PYRUVIC ACID AS AN INTERMEDIARY
METABOLITE IN THE BRAIN TISSUE OF AVITA-
MINOUS AND NORMAL PIGEONS.

BY RUDOLPH ALBERT PETERS and
ROBERT HENRY STEWART THOMPSON.

From the Department of Biochemistry, Oxford.

(Received May 4th, 1934.)

The recent discovery of a pyruvate reaction in lactate solutions in which avitaminous pigeon's brain has respired *in vitro* for 2 hours [Peters and Sinclair, 1933] is of interest in relation to the action of vitamin B₁, because addition of this reduces the amount of the abnormal substance (for references see Gavrilescu *et al.* [1932] and Meiklejohn *et al.* [1932]). More generally, identification of pyruvate in these circumstances lends support from an unexpected source to the recent conceptions of this substance as a normal intermediary in the metabolism of carbohydrate in the animal (see especially Embden *et al.* [1933] and Meyerhof and Kiessling [1933]). Otherwise these views depend entirely upon work with added sulphite or fluoride. There is also involved the even more fundamental problem of synthesis of carbohydrate from lactic acid, since the indication is that vitamin B₁ is related to the oxidative synthesis of lactate. With this idea in mind, we have devoted attention in the main to phenomena occurring in lactate solutions, as we are more likely so to approximate to conditions favouring the synthesis. The experiments described in this paper have been made with minced brain and in phosphate solutions to facilitate manipulations and to obtain better duplicate control. The conditions are frankly artificial and narrowed for

the study of the one point, but it is most unlikely that qualitative errors have been imposed by our conditions. The presence of the substance reacting like pyruvate is equally induced by sections of avitaminous brain and was found by Sinclair (personal communication) to occur with bicarbonate solutions.

We have proved that the nitroprusside-reacting substance is actually pyruvic acid and have obtained quantitative data relating extra pyruvate disappearance in presence of vitamin to extra oxygen uptake to find out whether pyruvate disappears as a direct or indirect effect of the vitamin.¹

o o o o

Pyruvate as substrate.

The conclusion that the vitamin catalyses disappearance of pyruvate as well as increased O₂ uptake can be confirmed by substitution of pyruvate for lactate in the respiring system. This is in striking contrast to lactate, extra disappearance of which was not shown to take place in presence of vitamin [Meiklejohn, 1933].

o o o o

Pyruvic acids is the first organic substance which has been proved to disappear *in vitro* as the result of action of vitamin B₁, this change accompanying the extra oxygen uptake. Much of the evidence presented up to this point is consistent with the idea that the vitamin acts directly upon the pyruvate.

o o o o

SUMMARY.

1. Disappearance pyruvic acid and of bisulphite-binding substances accompanies the extra oxygen uptake induced by the action of crystalline vitamin B₁ in pigeon's brain tissue respiring *in vitro*. Estimations have been made by the method of Clift and Cook and by a modified Case method.

2. Pyruvate also disappears when substituted for lactate.

3. The average value for the quotient extra O₂ uptake/pyruvic acid disappearing approximates to 506, representing 2 mols. O₂ taken up to 1 mol. pyruvic acid disappearing, but there are wide variations, which lead to the belief that disappearance of pyruvate is an indirect effect of vitamin action.

4. The phenomena are consistent with the view that pyruvic acid is a normal intermediary in the metabolism of pigeon's brain tissue. In agreement with the Embden-Meyerhof fermentation scheme, it accumulates with respiring normal brain tissue in presence of iodoacetate and not of fluoride.

ANEMIA OF INFLAMMATION AND THE ROLE OF THE RETICULOENDOTHELIAL CELLS

The hypoferrremia in inflammation is due to shortened disappearance time of serum iron and the failure of RE cells to release iron.

Key Words: iron, anemia, inflammation, reticuloendothelial cells

The anemia which commonly complicates chronic infections and chronic inflammatory disorders is characterized by low serum iron, a low iron-binding capacity of serum, and increased tissue iron stores.¹ The widely held opinion is that the low serum iron concentration is due to the failure of the reticuloendothelial (RE) cells to release iron. Although impaired intestinal absorption was also considered to be a causative factor, its contribution was believed not to be significant.² With the development of more refined techniques employing radioactive isotopes, the interpretation of studies on iron metabolism has become much easier. Employing some of these recent methods, Hershko and co-workers³ attempted to study the contribution of these two mechanisms to the hypoferrremia of inflammation.

Inflammation was produced in eight-week-old rats by intramuscular injections of turpentine. Within five hours following the injection, a profound fall in serum iron and total iron-binding capacity was observed and the effect was evident even 24 hours later. Plasma iron turnover was studied by injecting transferrin-bound ⁵⁹Fe, which was prepared by incubating rat plasma in vitro with ⁵⁹Fe. The disappearance half-time was considerably shortened to 48 minutes, as against 93 minutes in the control animals. The authors consider this to be an important cause of hypoferrremia. Since this group had earlier demonstrated⁴ that animals in this age group

derive nearly 60 percent of their serum iron from the intestinal cells, intestinal iron absorption was next studied.

The animals were given food extrinsically labeled with ⁵⁹FeCl₃ by the methods described by Cook and his co-workers⁵ and by Hallberg and Björn-Rasmussen.⁶ This technique considerably simplified the study of iron absorption. Whole body radioactivity in these animals was counted 24 hours and five days later and iron absorption was calculated. There was a significant reduction in iron absorption, the reduction being much more than could be explained by the reduced food intake, a common feature of all inflammatory conditions. The iron obtained through intestinal absorption, however, still constituted nearly 55 percent of the total serum iron.

The release of iron by the RE cells was then studied by a double isotope procedure. Most of the iron entering the RE cells is in the form of intracellular hemoglobin; extracellular hemoglobin bound to haptoglobin is taken up by the parenchymal cells.⁷ The animals were given simultaneous injections of heat-damaged erythrocytes labeled with ⁵⁹Fe and of transferrin-bound ⁵⁵Fe. Blood radioactivity was counted over a period of ten days and the release of iron by RE cells calculated. For the study of parenchymal cell activity, ⁵⁹Fe hemoglobin-haptoglobin was used instead of labeled erythrocytes.

Total hepatic radioactivity was found to be high in inflammation. The radioactivity in heme was markedly low with a recipro-

cal increase of radioactivity in ferritin. There was a definite decrease in the release of iron, the defect being more evident in RE cells and the percent release being approximately one-half of the control values. This effect was more pronounced in the early release of iron.

The authors interpret these observations as indicative of a synchronous effect of inflammation on intestinal cells, RE cells, and hepatocytes. Due to the increased breakdown of erythrocytes, more iron would enter the RE cells resulting in increased ferritin concentration, but with a defect in the release of iron into circulation leading ultimately to anemia. □

1. A. J. Erslev in *Anemia of Chronic Disorders in Hematology*. W. J. Williams, E. Beutler, A. J. Erslev, and R. W. Rundles, Editors, pp. 371-380. McGraw-Hill Book Co., New York, 1972

2. G. E. Cartwright and M. M. Wintrobe: The Anemia of Infection. XVII. A Review. *Adv. Int. Med.* 5: 165-226, 1952
3. C. Hershko, J. D. Cook, and C. A. Finch: Storage Iron Kinetics. VI. The Effect of Inflammation on Iron Exchange in the Rat. *Brit. J. Haemat.* 28: 67-75, 1974
4. J. D. Cook, C. Hershko, and C. A. Finch: Storage Iron Kinetics. V. Iron Exchange in the Rat. *Brit. J. Haemat.* 25: 695-706, 1973
5. J. D. Cook, M. Layrisse, C. Martinez-Torres, R. Walker, E. Monsen, and C. A. Finch: Food Iron Absorption Measured by an Extrinsic Tag. *J. Clin. Invest.* 51: 805-815, 1972
6. L. Hallberg and E. Björn-Rasmussen: Determination of Absorption from Whole Diet. A New Two-Pool Model Using Two Radioiron Isotopes Given as Haem and Non-Haem Iron. *Scandinav J. Haemat.* 9: 193-197, 1972
7. C. Hershko, J. D. Cook, and C. A. Finch: Storage Iron Kinetics. II. The Uptake of Hemoglobin Iron by Hepatic Parenchymal Cells. *J. Lab. Clin. Med.* 80: 624-634, 1972

REGULATION OF LIVER METABOLISM OF PYRIDOXAL PHOSPHATE

That formation of pyridoxal phosphate from pyridoxine is regulated in vivo is suggested by the fact that high dietary intakes of pyridoxine do not increase brain and liver pyridoxal phosphate in rats. Also, the pyridoxal phosphate concentration of isolated rat hepatocytes remains constant even when high concentrations of pyridoxine are added to the medium. Inhibition of cellular phosphatases, however, results in increased pyridoxal phosphate formation from pyridoxine added to the medium. Much of the pyridoxal phosphate synthesized becomes bound to protein. The protein-bound pyridoxal phosphate is less susceptible to hydrolysis.

Key Words: pyridoxal phosphate (PLP), apoenzymes, binding proteins, regulatory mechanism

Vitamin B₆ occurs in liver chiefly as pyridoxal phosphate (PLP) and pyridoxamine phosphate (PMP).^{1,2} The level of vitamin B₆ in rat liver increases with the dietary intake of pyridoxine up to 25 to 50 µg per day, but no additional increase in liver vitamin B₆ occurs with higher intakes.^{3,4} In contrast, at very high pyridoxine intakes, a reduction in total vitamin B₆ concentration in liver has been found, with statistically significant decreases occurring in PLP and PMP.²

The fact that high intakes of pyridoxine or other B vitamins do not lead to higher tissue levels of that particular vitamin may be considered evidence for regulatory mechanisms in vivo. A regulatory mechanism for PLP formation would appear to be needed in view of the known reactivity of PLP with various enzymes in vitro, together with the possibility that such reactions might occur in vivo.⁵ At present, relatively little is known about the factors regulating tissue levels of PLP although recent evidence indicates that the rate of dissociation of PLP from its apoenzymes may be a factor in determining the breakdown of the

enzyme protein.⁶ In rats with vitamin B₆ deficiency, specific protease enzymes may be involved in the degradation of these apoenzymes.^{7,8}

It has been suggested that pyridoxine phosphate oxidase or various cellular phosphatases may be the regulators.^{9,10} Pyridoxine phosphate oxidase catalyzes the oxidation of pyridoxine phosphate (formed from dietary pyridoxine) to pyridoxal phosphate.⁹ This enzyme is quite sensitive to inhibition by its product PLP. This sensitivity might be useful in shutting down formation of additional PLP when tissue levels of PLP are high. With respect to the phosphatases, several of these enzymes can catalyze the hydrolysis of PLP and can act on other phosphate compounds as well.¹⁰

Li and co-workers¹¹ investigated the metabolism of PLP in rat liver and in isolated rat hepatocytes, in order to obtain more information on the regulation of PLP metabolism. Male, weanling Sprague-Dawley rats were fed ad libitum a liquid pyridoxine-deficient diet supplying 0.5, 5.0, or 50 μg per milliliter of pyridoxine. The composition of this diet was not given, except for the commercial name of the diet and its supplier. The authors stated, however, that growth was "normal" with this diet, although they did not give weight gains in the 20-day period. The rats ate, however, 25, 250, and 2500 μg of pyridoxine per day, values which correspond to 1/3, 3.6, and 36 times the daily requirement of the rat.¹²

These large differences in pyridoxine intake produced no significant differences in the levels of PLP in the liver or in the brain. With all three diets, the liver values were 39 to 40 nmoles per gram of wet weight, and the brain values were between 7.7 and 8.1 nmoles per gram of wet weight. The value of 7.7 occurred with the group receiving the 25 μg daily intake of pyridoxine. This value, however, was not significantly lower than the values for the other two groups.

Experiments were done with isolated hepatocytes to test whether increasing the pyridoxine concentration in the incubation

medium could increase the intracellular concentration of PLP. Hepatocytes were isolated from livers of male rats weighing 150 to 180 g, which had been fasted for 24 hours. These livers were perfused with collagenase, minced, and treated with hyaluronidase. The viability of these cells was satisfactory, as indicated by exclusion of trypanblue, and their capacity for gluconeogenesis from pyruvate was 15 μmoles per hour per 100 mg of protein. The incubation medium (5 ml) contained 2.6 mM or 80 mM sodium phosphate, with KCl, MgSO_4 , glucose, bovine serum albumin, and NaCl, to bring the osmolarity to 290 mOsm. Hepatocytes representing 12 mg of protein were used. The cells incubated in the 2.6 mM phosphate medium (a physiological concentration of phosphate), showed no changes in cellular PLP concentration throughout a 45 minute incubation, as the pyridoxine concentration in the medium was increased up to 500 μM .

Other work by Li and co-workers showed that the erythrocyte content of PLP could be increased by inhibiting phosphatase activity, when pyridoxine or pyridoxal were the substrates added. The importance of phosphatase inhibition in regulating PLP levels was shown by the increase in hepatocyte PLP when the cells were incubated in the presence of 80 mM phosphate, which inhibits phosphatase activity. Cells incubated in 80 mM phosphate showed increases in cellular PLP throughout the incubation as the pyridoxine concentration of the medium increased from 0.5 to 5 μM , with an additional increase at 50 μM . No additional increases occurred when the pyridoxine concentrations were greater than 50 μM .

When the cells were incubated in the 80 mM phosphate medium without added pyridoxine, no change occurred in cellular PLP concentration during the incubation. Similar results were obtained when pyridoxal, rather than pyridoxine, was added to the incubation medium.

The lack of increase with the 2.6 mM phosphate medium, despite the high concentrations of pyridoxine in the medium, is

evidence that cellular mechanisms exist for regulation of PLP formation, in the presence of an excess vitamin precursor.

Synthesis of PLP by the liver cytosol was then investigated to obtain information on the potential role of pyridoxine phosphate oxidase in the regulation of liver PLP levels. When pyridoxine and ATP were added to cytosol preparations, the concentration of PLP increased rapidly. After two hours of incubation, the PLP concentration was ten times higher than that found initially in the cytosol before incubation. Net synthesis of PLP from added pyridoxine still occurred to the same extent, even when PLP (50 μ M) was added to the incubation mixture at the start of the incubation. This concentration (50 μ M) of PLP is greater than the concentration present in the original liver. Under these circumstances, the concentration of PLP reached in the system increased by nearly the same amount as before. Some destruction of PLP still occurred, presumably because of incomplete inhibition of the phosphatases, even at 0.2 M phosphate in the medium.

Thus, under these conditions, PLP did not appear to have inhibited pyridoxine phosphate oxidase, although the levels of PLP added to the cytosol incubations would have been inhibitory with the purified enzyme. The lack of inhibition could have resulted from binding of the pyridoxal-phosphate to proteins in the cytosol, so that the concentration of "free" pyridoxal-phosphate remained low.

That PLP formed by cytosol preparations was bound to protein was indicated by experiments in which only 50 percent of the PLP could be removed from the cytosol by dialysis. Furthermore, the PLP remaining in the cytosol was resistant to hydrolysis by the phosphatase activity of liver plasma membrane preparations, although nonprotein-bound PLP was hydrolyzed. These results indicate that binding by proteins can act to protect cellular PLP, whereas phosphatase activity can be responsible for the degradation. Li and co-workers speculated that degradation would be rapid for any PLP formed in

excess of the binding capacity for the coenzyme.

No identification has yet been made of these PLP-binding proteins in liver. It is possible that the "binding" proteins may be apoenzymes from which the PLP has dissociated. Glycogen phosphorylase, for example, is the principal protein binding PLP in muscle.¹³ Exchange of PLP has been found to occur between different apoenzymes.¹⁴ Anion binding proteins also exist in liver, and these may be able to bind PLP.¹⁵ More information is needed on the mechanisms by which PLP is hydrolyzed by the liver. Initial data suggest that plasma membrane-associated alkaline phosphatases may be responsible.¹⁶

Experiments such as these emphasize how little is known about the factors which regulate the formation and degradation of coenzymes. The importance of information on this subject is emphasized by evidence indicating that coenzyme dissociation may determine the rate of turnover of apoenzyme protein. □

1. J. B. Lyon, Jr., J. A. Bain, and H. L. Williams: The Distribution of Vitamin B₆ in the Tissues of Two Inbred Strains of Mice Fed Complete and Vitamin B₆-Deficient Rations. *J. Biol. Chem.* 237: 1989-1991, 1962
2. P. A. Cohen, K. Schneidman, F. Ginsberg-Fellner, J. A. Sturman, J. Knittle, and G. E. Gaull: High Pyridoxine Diet in the Rat: Possible Implications for Megavitamin Therapy. *J. Nutrition* 103: 143-151, 1973
3. V. F. Thiele and M. Brin: Availability of Vitamin B₆ Vitamers Fed Orally to Long-Evans Rats as Determined by Tissue Transaminase Activity and Vitamin B₆ Assay. *J. Nutrition* 94: 237-242, 1968
4. G. H. Beaton and M. C. Cheney: Vitamin B₆ Requirement of the Male Albino Rat. *J. Nutrition* 87: 125-132, 1965
5. S. Shapiro, M. Enser, E. Pugh, and B. L. Horecker: The Effect of Pyridoxal Phosphate on Rabbit Muscle Aldolase. *Arch. Biochem.* 128: 554-562, 1968
6. G. Litwack and S. Rosenfield: Coenzyme Dissociation, A Possible Determinant of Short Half-Life of Inducible Enzymes in Mammalian Liver. *Biochem. Biophys. Res. Commun.* 52: 181-188, 1973

7. A Specific Protease for Pyridoxal Enzymes. *Nutrition Reviews* 31: 98-99, 1973
8. Control of Enzyme Levels in Vitamin Deficiency. *Nutrition Reviews* 30: 232-234, 1972
9. G. M. Brown and J. J. Reynolds: Biogenesis of the Water-Soluble Vitamins. *Ann. Rev. Biochem.* 32: 419-462, 1963
10. J. M. Turner: Pyridoxal Phosphate Breakdown by an Alkaline Phosphatase Preparation. *Biochem. J.* 80: 663-668, 1961
11. T. K. Li, L. Lumeng, and R. L. Vietsch: Regulation of Pyridoxal 5'-Phosphate Metabolism in Liver. *Biochem. Biophys. Res. Commun.* 61: 677-684, 1974
12. *Nutrient Requirements of Laboratory Animals*. Second revised edition, pp. 80-81. National Academy of Sciences/National Research Council, Washington, D. C., 1972
13. E. H. Fischer, A. Pocker, and J. C. Sarri: The Structure, Function, and Control of Phosphorylase. *Essays Biochem.* 6: 23-68, 1970
14. J. E. Churchich: Cofactor Transfer from Cystathionase to Aspartate Aminotransferase. *Biochem. Biophys. Res. Commun.* 40: 1374-1379, 1970
15. A. J. Levi, Z. Gatmaitan, and I. M. Arias: Two Hepatic Cytoplasmic Protein Fractions, Y and Z, and Their Possible Role in the Hepatic Uptake of Bilirubin, Sulfobromophthalein, and Other Anions. *J. Clin. Invest.* 48: 2156-2167, 1969
16. T. K. Li and L. Lumeng: Regulation of Hepatic Pyridoxal Phosphate Content: A Role of Alkaline Phosphatase. *Fed. Proc.* 33: 1546, 1974

28 NOV 1975

NEUROLOGICAL DAMAGE IN VITAMIN B₁₂-DEPLETED BATS

Fruit bats fed an all fruit diet for 200 days or longer became depleted of vitamin B₁₂. They exhibited neurological damage, characterized by ataxia with difficulty in climbing and abnormalities in the down stroke of the flight cycle. Histological examination of the spinal cord revealed changes indicative of demyelination. The similarity of the neurological changes to those seen in advanced pernicious anemia suggests that the bat may provide a useful animal model for studying this aspect of the disease.

Key Words: pernicious anemia, vitamin B₁₂ deficiency, spinal cord demyelination, propionic acid metabolism, fruit bats, neurological abnormalities

In man, pernicious anemia results from impaired absorption of vitamin B₁₂. It manifests itself primarily in a megaloblastic anemia and subacute combined degeneration (demyelination) of the spinal cord.

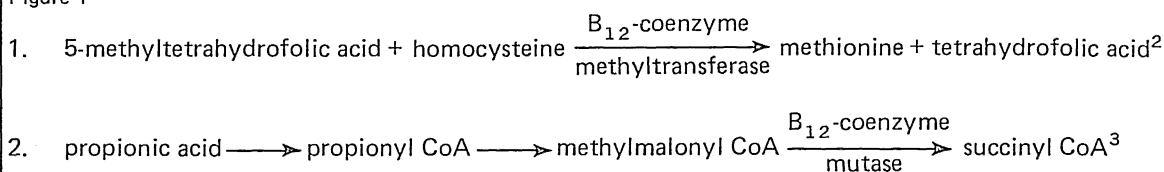
Common experimental animals such as the rat and the chick require vitamin B₁₂ for normal growth, reproduction and egg hatchability. They have been of limited usefulness in studying the pathophysiology of the hematological and neurological disturbances, however, because vitamin B₁₂ deficiency does not produce anemia or consistent neurological symptoms comparable to those in human beings.¹

Biochemically, vitamin B₁₂ is known to function as a coenzyme in only two reactions in mammals. (See Figure 1).

In the first reaction vitamin B₁₂ coenzyme is required for the methylation of homocysteine to form methionine. The methyl group is contributed by 5-methyl-tetrahydrofolic acid and tetrahydrofolic acid is regenerated. In the second reaction, propionic acid is converted to propionyl CoA, then to methylmalonyl CoA and in the presence of vitamin B₁₂ coenzyme and a mutase, it is isomerized to succinyl CoA for metabolism in the citric acid cycle.

Morphologically, the megaloblastic anemia of pernicious anemia is indistinguishable from that of a folic acid deficiency. Since large doses of folic acid

Figure 1



restore the megaloblastic state to normal, it has been suggested⁴ that in pernicious anemia, the vitamin B₁₂ deficiency produced a secondary deficiency of folic acid. A mechanism proposed is that the vitamin B₁₂ methyltransferase reaction may be impaired by the vitamin B₁₂ deficiency and folate thus becomes "trapped" as 5-methyltetrahydrofolate because of the essentially irreversible methylenetetrahydrofolate reductase reaction. Supplementing folic acid would bypass this reaction and provide the folate derivatives needed for essential purine and pyrimidine synthesis and normal erythropoiesis.

More recent studies suggested that the "methyl trap" theory may have to be modified to also include a diminished folate transport and a reduced synthesis of the polyglutamate forms of folic acid in vitamin B₁₂-deficient animals.⁵

Folic acid does not prevent the neurological disturbances of pernicious anemia. Therefore, investigations and theories for the biochemical lesion⁸ causing the neuropathy centered on various aspects of deranged propionic acid metabolism.^{6,7} The lack of an experimental animal model, however, hindered progress in this area also. A report⁸ that captive monkeys fed a vegetarian diet develop a cage paralysis resembling the demyelination of pernicious anemia, suggested that these animals might provide a suitable experimental model. The results, however, have not been confirmed^{9,10} and the exact relation of cage paralysis in monkeys and vitamin B₁₂ deficiency remains unclear.

Another approach to this problem has been reported by Green et al.,¹¹ who studied vitamin B₁₂ deficiency in the tropical fruit bat (*R. aegyptiacus*). This species was chosen because it is easy to raise in

captivity and its natural diet consists mainly of fruits and flowers. Coprophagy has not been observed in these animals, thus minimizing the chances of fecal vitamin B₁₂ contamination. It is thought that in its natural state, the bat gets its vitamin B₁₂ from inadvertent ingestion of fruit insects and by drinking stagnant water. By excluding these sources of the vitamin, a natural fruit diet, free of vitamin B₁₂, could be fed the bats for long periods of time.

Washed, pest-free all-fruit diets consisting of peeled bananas, papayas, pears and oranges were fed to adult bats. Clean tap water was supplied daily. At various times after being placed on the diet, animals were sacrificed and concentrations of vitamin B₁₂ in blood and liver analyzed. Stained slides of rib bone marrow were prepared, as were spinal cords from freshly sacrificed animals which were stained for detection of myelin and axons.

At time of capture, average serum vitamin B₁₂ concentration of the bats was 1964 ± 121 pg ml⁻¹. After 330 days on the fruit diet, it had dropped to 98.6 ± 35 pg ml⁻¹. During the same period, liver vitamin B₁₂ concentrations also fell significantly. The hemoglobin, hematocrit and red cell count, however, were not altered by the deficiency and there was no sign of megaloblastic change in the bone marrow. Leukocyte count was significantly depressed by the deficiency (19.5 to 4.9 thousand μ l⁻¹) after 130 days on diet. The injection of 200 ng of vitamin B₁₂ per week in two bats restored leukocyte concentrations to initial values, indicating the changes were due to the vitamin depletion.

As the bats became more deficient (after 200 days on diet), seven out of ten surviving animals developed ataxia and had

difficulty climbing. There seemed to be a loss of reflex proprioceptive sensation in their feet. They also had trouble disengaging their claws from the wire mesh.

Flight cycle characteristics of the animals were also analyzed by high speed cine and still photography. Abnormalities in the downstroke part of the cycle were observed in deficient animals. Wing surfaces were crumpled and irregular compared with the normally smooth, extended wing of the controls. The usually fully extended phalanges trailed in the slip stream of deficient bats.

Spinal cord sections, obtained at 200 days from four out of five bats exhibiting the climbing and flying abnormalities, showed patchy, spongiose change in the white matter of the lower cervical and upper thoracic regions. This mainly affected the lateral and ventrolateral columns and resembled early demyelination. The spinal cord lesions were similar to those seen in pernicious anemia, except that in the human lesion, the dorsal and lateral columns were mainly affected. Bats given weekly, intramuscular injections of 200 ng cyanocobalamine did not develop neurological changes. Once the functional changes had appeared, however, vitamin B₁₂ failed to reverse them.

This study showed that a relatively easy to care for animal, the bat, could be depleted of vitamin B₁₂ by feeding its natural fruit diet. The blood picture presented by the vitamin B₁₂-depleted bat resembled that seen in the vitamin B₁₂-depleted rat¹ and would not serve as a model for studying the megaloblastic anemia of pernicious anemia. The folic acid provided by the fruit may have prevented the hematological changes. The vitamin B₁₂-depleted bat appears to provide, however, a useful model for successfully inducing neurological changes that closely resemble the subacute combined degeneration of the spinal cord seen in pernicious anemia. If this model turns out to be as promising as it appears, various hypotheses on the biochemical lesion concerned with impaired

propionic acid metabolism and the incorporation of abnormal fatty acids into myelin membranes^{6,7,12} can be subjected to direct investigation. □

1. E. L. R. Stokstad: Experimental Anemias in Animals Resulting from Folic Acid and Vitamin B₁₂ Deficiencies. *Vitamin Hormones* 26: 443-463, 1968
2. W. Sakami and I. Ukstins: Enzymatic Methylation of Homocysteine by a Synthetic Tetrahydrofolate Derivative. *J. Biol. Chem.* 236: PC 50-51, 1961
3. E. V. Cox and A. M. White: Methylmalonic Acid Excretion: An Index of Vitamin B₁₂ Deficiency. *Lancet* II: 853-856, 1962
4. V. Herbert and R. Zalusky: Interrelations of Vitamin B₁₂ and Folic Acid Metabolism: Folic Acid Clearance Studies. *J. Clin. Invest.* 41: 1263-1276, 1962
5. Folic Acid Metabolism in Vitamin B₁₂ Deficiency. *Nutrition Reviews* 33: 118-120, 1975
6. G. Cardinale, T. J. Carty and R. H. Abeles: Effect of Methylmalonyl Coenzyme A, A Metabolite which Accumulates in Vitamin B₁₂ Deficiency, on Fatty Acid Synthesis. *J. Biol. Chem.* 245: 3771-3775, 1970
7. Y. Kishimoto, M. Williams, H. W. Moser, C. Hignite and K. Biemann: Branched-Chain and Odd-Numbered Fatty Acids and Aldehydes in the Nervous System of a Patient with Deranged Vitamin B₁₂ Metabolism. *J. Lipid Res.* 14: 69-77, 1973
8. C. E. Oxnard and W. T. Smith: Neurological Degeneration and Reduced Serum Vitamin B₁₂-Levels in Captive Monkeys. *Nature* (London) 210: 507-509, 1966
9. R. C. Siddons: The Experimental Production of Vitamin B₁₂ Deficiency in the Baboon (*Papio cynocephalus*). A 2-Year Study. *Brit. J. Nutrition* 32: 219-228, 1974
10. J. A. Kark, M. Victor, J. D. Hines and J. W. Harris: Nutritional Vitamin B₁₂ Deficiency in Rhesus Monkeys. *Am. J. Clin. Nutrition* 27: 470-478, 1974
11. R. Green, S. V. van Tonder, G. J. Oettle, G. Cole and J. Metz: Neurological Changes in Fruit Bats Deficient in Vitamin B₁₂. *Nature* 254: 148-150, 1975
12. F. W. Barley, G. H. Sato and R. H. Abeles: An Effect of Vitamin B₁₂ Deficiency in Tissue Culture. *J. Biol. Chem.* 247: 4270-4276, 1972

FDA Proposals Regarding Vitamin and Mineral Products

The Food and Drug Administration recently proposed to revise its 1973 regulations governing the formulation and labeling of vitamin and mineral products. Under the proposed revision, published in the Federal Register of May 28, 1975, high potency vitamin or mineral products generally recognized as safe and marketed as dietary supplements will be classified and regulated as food.

The original regulations had required that all preparations providing more than 150 percent of the United States Recommended Daily Allowance of any vitamin or mineral must be regulated as drugs rather than as food. FDA took the position that vitamins and minerals at doses higher than 150 percent of the U.S. RDA might be appropriate for treating vitamin-mineral deficiencies or for other therapeutic purposes, but were beyond reasonable limits of usefulness as dietary supplements for healthy individuals.

The U.S. Court of Appeals in New York City subsequently ruled that those high potency vitamins and minerals which are generally recognized as safe for consumer use, and for which no therapeutic benefits are claimed, could not be defined by FDA as drugs on the basis of potency alone. It,

therefore, directed the FDA to seek another approach to the regulation of such preparations. The Court did not contest and FDA has not changed its basic requirement that any vitamin or mineral product for which any therapeutic claim is made shall be deemed a drug.

The proposal includes the following:

(1) The FDA will permit the sale of dietary supplements consisting of a single vitamin or mineral at any potency generally recognized as safe. The earlier regulations had generally limited the potency of such products to 150 percent of the U.S. RDA.

(2) The FDA will consider applications for the marketing of combinations of vitamins and minerals which differ from those already permitted by the Agency. Combination products presently permitted are those which contain: vitamins only, minerals only, vitamins and minerals in combination, or vitamins plus iron. All vitamins and minerals in this context refer to the 19 essential nutrients for which U.S. RDA's have been established.

(3) The FDA will reopen the vitamin-mineral hearing to permit examination of one witness on the development of the U.S. RDA system.

The National Advisory Committee on Hyperkinesis and Food Additives

Aware of the anxiety created by the publicized hypothesis advanced by Dr. Ben F. Feingold in 1973 implicating many widely distributed or highly nutritious food-stuffs and undesignated food additives as potential factors in hyperkinesis, The Nutrition Foundation has supported a responsible, critical review by a competent group of expert medical and behavioral scientists of

evidence relative to this hypothesis. The scientists who comprised the review committee were selected and identified by the offices of major scientific and medical organizations: American Medical Association's Council on Foods and Nutrition; American Psychiatric Association; American Association of Child Psychiatry; Society of Toxicology; Council for Excep-

tional Children; American Alliance for Health, Physical Education; Institute of Food Technologists; American Society for Clinical Nutrition; American Dietetic Association; The National Nutrition Consortium; Life Sciences Research Office of the Federation of American Societies for Experimental Biology; Committee on Nutrition of the American Academy of Pediatrics; and the Food and Nutrition Board of the National Academy of Sciences.

The Committee was charged with the responsibility to (a) review critically and objectively the nature of evidence relative to the hypothesized relationship, (b) recommend whether additional investigations were justified or desirable, and, if so, (c) provide guidelines for the experimental design of appropriate studies which would result in obtaining valid data upon which conclusions might be formulated. The background of the committee is such as to provide valuable guidance in assessing evi-

dence and design of any subsequently projected clinical and experimental investigations of the postulated relationship between hyperkinesis and food.

Dr. Morris Lipton, Professor of Psychiatry, University of North Carolina and a member of the American Psychiatric Association is Chairman of the Committee.

The Committee concludes that data from critically designed and executed studies, free of the deficiencies of design, must be available before firm conclusions can be reached on the Feingold hypothesis. Accordingly, the Committee has considered at length the experimental design characteristics necessary to obtain definitive, interpretable data which may permit a decisive interpretation.

Copies of the 20 page report are available from:

The Nutrition Foundation
Office of Education and Public Affairs
888 Seventeenth Street, N.W.
Washington, D.C. 20006

Anticonvulsant Drugs and Calcium Metabolism

Recent studies indicate that vitamin D (calciferol) must undergo two enzymatic hydroxylations before it can function at target cells. Thus, calciferol is transported from the skin or gastrointestinal tract to the liver, where it is converted to 25-hydroxycalciferol. After hydroxylation by the liver, 25-hydroxycalciferol is transported to the kidneys, where it undergoes a second hydroxylation to 1,25-dihydroxycalciferol, which is currently thought to be the final active metabolite of vitamin D. Current evidence suggests that anticonvulsant drugs may disrupt this normal sequence by inducing hepatic enzymes that increase the catabolism of vitamin D and its biologically active products.

Although anticonvulsant drugs have been widely prescribed for many years, their

adverse effect on vitamin D metabolism was not suspected until the late 1960's, when reports from Germany implicated these agents in the development of biochemical and radiologic signs of rickets.^{1,2} Subsequent reports confirmed these initial observations. Thus, several surveys of large groups of epileptic patients receiving anticonvulsant medications revealed a 10 to 30 per cent frequency of depressed serum calcium and elevated serum alkaline phosphatase concentrations.³⁻⁶ In most patients the biochemical abnormalities were mild and subclinical, but there are several reported cases of overt rickets and osteomalacia attributable to anticonvulsant drugs.^{7,8}

Because most of the surveys were conducted on institutionalized populations, it was thought that the alterations of calcium and bone metabolism might, in part, be related to lack of sunlight exposure, in-

sufficient physical activity or suboptimal dietary vitamin D. The observations of Lifshitz and Maclaren are pertinent in this regard.⁶ In their study of institutionalized, mentally retarded children receiving anticonvulsant medications and 800 to 1200 IU of vitamin D per day, biochemical and roentgenologic evidence of rickets was found only in non-ambulatory patients who were confined indoors and in whom chronic recurrent infections were common. Moreover, Richens and Rowe, in studies of patients receiving anticonvulsant drugs at a residential center, found that low serum calcium levels were much more prevalent in patients who worked indoors than in those engaged in outdoor work.⁹ Observations of this nature, and the fact that two reported studies of juvenile epileptic outpatients are at variance,^{2,10} have generated uncertainty regarding the susceptibility of non-institutionalized children to anticonvulsant-induced abnormalities of calcium metabolism.

The study of Hahn et al* reported in this issue of the *Journal* is timely and demonstrates that in ambulatory outpatient juvenile epileptic patients, chronic treatment with phenobarbital or diphenylhydantoin (or both) is associated with a significant reduction in bone mass as measured by photon absorptiometry, as well as with significant reductions in serum 25-hydroxycalciferol and serum calcium. The vitamin D intake of these children was adequate by current standards and averaged 3500 to 3860 IU per week. From a practical standpoint it is of interest that there were no significant alterations in the serum phosphorus and that the differences between the mean serum calcium in the control and in the anticonvulsant-treated populations, although significant, were slight; only two of 50 anticonvulsant-treated children had serum calcium levels below the normal range. In most clinical practices serum 25-hydroxycalciferol assays and photon absorptiometry are not avail-

able, and it might therefore be extremely difficult, if not impossible, to diagnose an existing altered state of calcium metabolism in an active and otherwise healthy child receiving anticonvulsant medications. It seems likely that if the child is receiving 400 to 600 IU of vitamin D per day and is exposed to sunlight, as in the study of Hahn et al., there will be no pathologic alterations in serum calcium and phosphorus, and standard roentgenographs of bone may be normal, whereas serum alkaline phosphatase may be mildly to moderately elevated. A possible exception is the anticonvulsant-treated child who, for reasons as yet unknown, but perhaps owing to individual host susceptibility, presents with overt biochemical and radiologic signs of rickets. The value of the study of Hahn et al. is that, through careful investigation and the use of recently developed procedures, a disorder of calcium and vitamin D metabolism was detected that might not be uncovered with the diagnostic tools available in most clinical settings. An equally careful study to determine whether the decrease in bone mass in anticonvulsant-treated children is associated with alterations of linear growth would be of interest.

Hahn et al. point out that a combination of drug therapy (phenobarbital and diphenylhydantoin) was associated with the most marked alterations in serum calcium, serum 25-hydroxycalciferol and bone mass, and suggest that the vitamin D intake in these children should be increased to at least 10,000 IU per week. Although an increase in vitamin D supplementation seems reasonable, further studies and observations are needed for better quantification of the vitamin D needs of the children receiving anticonvulsant drugs. Any prophylactic program in which an increase in vitamin D is prescribed should be administered with caution, and appropriate measures taken to avoid vitamin D toxicity.

The disorder in calcium and vitamin D metabolism produced by anticonvulsant therapy presents a challenge, since in most

*New Engl. J. Med. 292: 550-554, 1975

patients discontinuation of anticonvulsant therapy is medically contraindicated. Further work is needed to clarify the role of a number of factors in this disorder, including drug dosage, duration of treatment, vitamin D intake, sunshine exposure, physical activity, parathyroid function, infections and individual host susceptibility. In addition, further studies are required to define better the mechanism whereby chronic anticonvulsant therapy produces abnormalities in vitamin D and calcium metabolism. As more knowledge is acquired, a better guide to prophylaxis and therapy with vitamin D or its metabolites should emerge.

Constantine S. Anast, M.D.
University of Missouri
Medical Center
Columbia, Missouri 65201

6. F. Lifshitz and N. K. Maclaren: Vitamin D-dependent rickets in institutionalized, mentally retarded children receiving long-term anticonvulsant therapy. I. A survey of 288 patients. *J. Pediatr.* 83: 612-620, 1973
7. C. E. Dent, A. Richens, D. J. F. Rowe, et al: Osteomalacia with long-term anticonvulsant therapy in epilepsy. *Br. Med. J.* 4: 69-72, 1970
8. A. D. Borgstedt, M. F. Bryson, L. W. Young, et al: Long-term administration of antiepileptic drugs and the development of rickets. *J. Pediatr.* 81: 9-15, 1972
9. A. Richens and D. J. F. Rowe: Anticonvulsant osteomalacia. *Br. Med. J.* 4: 684, 1971
10. S. Livingston, W. Berman and L. L. Pauli: Anticonvulsant drugs and vitamin D metabolism. *JAMA* 224: 1634-1635, 1973

1. F. Schmid: Osteopathien bei antiepileptischer Dauerbehandlung. *Fortschr Med.* 85: 381-382, 1967
2. R. Kruse: Osteopathien bei antiepileptischer Langzeittherapie. *Monatsschr Kinderheilkd* 116: 378-380, 1968
3. A. Richens and D. J. F. Rowe: Disturbance of calcium metabolism by anticonvulsant drugs. *Br. Med. J.* 4: 73-76, 1970
4. J. Hunter, J. D. Maxwell, D. A. Stewart, et al: Altered calcium metabolism in epileptic children on anticonvulsants. *Br. Med. J.* 4: 202-204, 1971
5. T. J. Hahn, B. A. Hendin, C. R. Scharp, et al: Effect of chronic anticonvulsant therapy on serum 25-hydroxycalciferol levels in adults. *N. Engl. J. Med.* 287: 900-904, 1972

Meeting Announcement

A three-day international symposium on NUTRITION AND DRUG INTERRELATIONS, sponsored by the Nutrition Foundation and the Iowa State University Nutrition Sciences Council, will be held on August 4-6, 1976 at Iowa State University. Major topics will be I. Drug Effects on Nutrient Intake, Function, and Requirement, II. Nutritional Effects on Drug Metabolism and Action, III. Drugs in Foods and Feeds, and IV. Nutrients and Foods as Drugs. For further information write Dr. John N. Hathcock, Department of Food and Nutrition, Iowa State University, Ames, Iowa 50010.

New from Academic Press

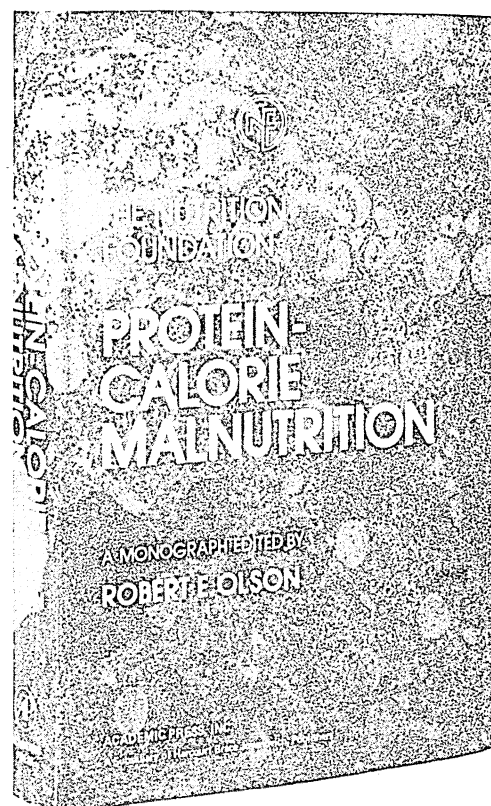
PROTEIN-CALORIE MALNUTRITION

A Nutrition Foundation Monograph

For physicians, nutritionists, public health practitioners, social workers, and medical students.

Covers all aspects of protein-calorie malnutrition (PCM), from molecular biology to epidemiology and prevention.

467 pages, \$29.50. Order from:
Academic Press, 111 Fifth Avenue,
New York, New York 10003
or your book supplier.



CONTENTS

Amino Acid Requirements and Age

Adaptation to Low-Protein Intakes

Protein Turnover in Various Nutritional States

Lysine Metabolism in Protein-Calorie Malnutrition with
Attention to the Synthesis of Carnitine

Cellular Energy Metabolism in Protein-Calorie Malnutrition

Brain DNA Synthesis in Protein-Calorie Malnutrition

Electroencephalograms, Learning, and Malnutrition

The Relationship of Severe Marasmic Protein-Energy
Malnutrition and Rehabilitation in Infancy to Subse-
quent Mental Development

Carbohydrate and Lipid Metabolism in Protein-Calorie
Malnutrition

Water-Soluble Vitamins in Protein-Calorie Malnutrition

Nutritional Survey of Children in the Dominican Republic
with Attention to Deficits in Water-Soluble Vitamins

The Fat-Soluble Vitamins and Protein-Calorie (Energy)
Malnutrition

Mineral Metabolism in Protein-Calorie Malnutrition

Mineral Metabolism in Protein Malnutrition

Endocrines and Malnutrition

Erythropoiesis in Protein-Calorie Malnutrition

The Anemia of Protein-Calorie Malnutrition

The Effect of Vitamin E on the Anemia of Protein-Calorie
Malnutrition in Northern Thai Children

The Effect of Variations in Protein and Calorie Intake on
the Rate of Recovery and Selected Physiological Re-
sponses in Thai Children with Protein-Calorie
Malnutrition

The Liver in Protein-Calorie Malnutrition: An Ultrastruc-
tural Study

Calories in the Treatment of Protein-Calorie Malnutrition

Protein-Sparing Effect of Excess Calories during Adapta-
tion to Low-Protein Intake

Protein versus Calories in the Treatment of Protein-Calorie
Malnutrition: Metabolic and Population Studies in India

Interactions of Malnutrition and Infection: Advances in
Understanding

Immunoglobulins and Complement in Protein-Calorie
Malnutrition

Cell-Mediated Immunity in Protein-Calorie Malnutrition

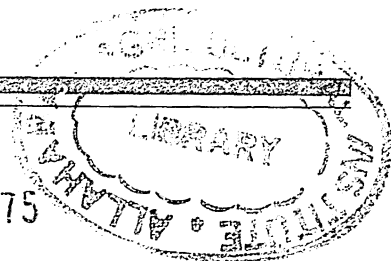
Treatment of Severe Protein-Calorie Malnutrition

The In-Patient and Out-Patient Treatment of the Child
with Severe Protein-Calorie Malnutrition

Prevention of Vitamin and Mineral Deficiencies Associated
with Protein-Calorie Malnutrition

Prevention of Protein-Calorie Malnutrition

11 NOV 1975



Nutrition, Food Needs and Technologic Priorities: The World Food Conference

by William J. Darby, M.D., Ph.D.

I propose to address four questions:

What is the nature of the World Food Crisis?

What was the World Food Conference in Rome, 5-15 November 1974?

What were its accomplishments?

What guidelines are there for determining priorities relative to meeting world food needs?

Several colleagues associated with The Nutrition Foundation participated in the World Food Conference. They included trustees of the Foundation: Dr. J. George Harrar; Mr. Henry J. Heinz II; Mr. J. E. Lonning; Rev. Theodore M. Hesburgh; The Nutrition Foundation's Senior Scholar, Dr. Samuel E. Stumpf; a member of the Food and Nutrition Liaison Committee, Dr. John Luck. I attended as a representative of the Non-Government Organizations (NGO). This summary draws heavily upon the resources of my colleagues. My selection of impressions and interpretations are not to be construed as necessarily representative of the views of this group of distinguished colleagues, however.

Dr. Darby is President of The Nutrition Foundation, Inc. This paper was presented at the Western Regional Conference on Higher Education for Fulbright-Hays Scholars at the University of Arizona, Tucson, Arizona on May 28, 1975.

A limited number of reprints of this article may be obtained from The Nutrition Foundation, 888 Seventeenth Street, N.W., Washington, D.C. 20006. THERE ARE NO REPRINTS OF UNSIGNED REVIEWS.

The Nature of the World Food Crisis

To the question, "Is there a world food crisis?" my reply is "yes." It has two dimensions. First, and most apparent, the dimension of famine—famine being an "extreme and general shortage of food causing distress and death from starvation among the population of a district or a country."¹ One needs only to reflect on recent events in Bangladesh, the Sahel and in Ethiopia to identify the famine dimension of the crisis.

Famine, its causes—drought, flood, destruction of a staple crop by microscopic parasite, invasion by locusts, consequences of war and civil disturbances, ill-conceived governmental action (e.g., Soviet collective farms)—and its avoidance are the subject of an authoritative book,¹ "The Conquest of Famine," by W. R. Aykroyd. This book, recently published by The Reader's Digest Press, and its author are the first recipients of the Nutrition Foundations' Book Award and provides strong documentation of the recurrence of such famines throughout history.

The second dimension of the world food crisis is that of the "impending" or temporarily averted general world food crisis resulting from the increasing disparity between population numbers and food production. The lack of balance between supplies and needs and the potential for fulfilling the needs were succinctly stated by Dr. George Harrar in his 1974 W. O. Atwater Lecture:²

Unfortunately, the world is substantially out of balance with respect to the produc-

tion and distribution of needed food supplies. It has long been recognized that there are many agrarian nations whose production levels are low and who regularly fail to satisfy their basic food requirements. Most successful agriculture is practiced in temperate climates, where soils are fertile, rainfall adequate and growing conditions, in general, favorable. Where the climate is harsh, water scarce, soils infertile and economic resources limited, agriculture tends to become a subsistence phenomenon.

Where there are complications caused by periodic floods, droughts or other natural disasters, the situation worsens. The Third World has long experienced the effects of an imperfect system of agricultural production and a long-term lag in economic development. Where this situation is compounded by explosive population growth, standards of living and the quality of life are woefully inadequate . . .

Presently, we are faced with new dilemmas, in combination with old ones. The agricultural industry is under increasing pressure to feed our present population plus those (200,000) who join the society every day. In a situation of energy crisis the problem becomes increasingly difficult and complicated, since agriculture basically depends to a very considerable degree on available energy. Adequate fertilizer, machinery, water systems and electrical power sources in concert make it possible to increase efficiency of agriculture with resultant production benefits. If one or more of those elements are subtracted from the system, reduction in the total food supply is an inevitable accompaniment. The lack of long-range and forward planning, the inability of nations to act in concert for the common good, instability of governments and the totally inadequate emphasis on food production has brought us to our present crisis now being exacerbated by energy constraints . . .

Fortunately, there is an enormous body of available information, a vast technology, improved biological materials, and an ever-growing cadre of qualified individuals who could give meaning and leadership to any national agricultural development plan. If each nation participates in a global effort to bring about the maximum efficient utilization of its agricultural and human resources for the production of food and agricultural

commodities, then it becomes possible to greatly increase total figures worldwide and to plan a system in which food production and distribution can be in harmony with human needs without the convulsions of crisis . . .

We can have a green revolution worldwide. Many of those who have written on this subject seemed to have failed to understand its true origins, meaning, and implication for the future. In fact, the Green Revolution, where it has been applied, has been a dramatic demonstration of the potential of combining all the elements of an efficient agricultural production system and translating them into greatly increased production figures. It has clearly demonstrated the fallacy of some of the earlier claims that many countries are doomed to hunger, disadvantage, and misery on a continuing basis because they are incapable of improving their most fundamental requirement, that of an adequate food supply and a proper human diet. The success of the Green Revolution could be repeated again throughout much of the world where both the need and the opportunity exist to apply its principles and practices.

It is reasonable to conclude that no country has maximally utilized all of the potential applications to food of the new technology. But it is apparent that the abundance of food and freedom from want are greatest in those countries where scientific technology has been most intensively developed and applied.

The Benefits of Technology

The impacts of this application of science are reflected in measurable indices of health. For example, in the United States the industrialization of food production at all levels—with concomitant awareness of nutritional needs and safety—has been accompanied by a virtual disappearance within this century of the classical deficiency diseases: pellagra, scurvy, rickets, endemic goiter, protein deficiency. Infant mortality, a widely employed index of nutritional health of a nation, reached an all time low in the U.S. in 1974. By contrast, in many regions where food production and distribution systems are

primitive, famine recurrently strikes and high death and morbidity rates continue because of pellagra, iodine deficiency goiter, beriberi, protein-calorie malnutrition, folic acid or iron deficiency anemia, ariboflavinosis, and vitamin A deficiency and resultant blindness. These exist in concert with severe and deadly food-borne infections—diarrheal diseases of the young child, enteric infestations, febrile illnesses and acute intoxications. It is commonplace for half of the children born to fail to survive until five years of age. All of these are indices of poor food and water sanitation and contributors to undernutrition and its sequelae.

Other health changes, the so-called diseases of the affluent society, may accompany industrialization. But these are not primarily due to alteration in food patterns. They reflect the profound differences in life styles between the rural non-industrialized society and the urbanized industrial complex and, in part, the increased survival time and longevity that results from scientific development. People live long enough to develop the diseases of the older adult. None of these diseases, however, are strangers to societies that are scientifically untouched. They occur, usually among the affluent elite, but are not major causes of illness or death in the total population. Modern application of scientific knowledge of health and food sciences and proper recognition of nutritional needs can reduce the toll of these diseases in developed regions.

During the earlier development of industrialized food systems some deficiency states arose, but these do not occur in the technologically developed countries in modern times because of the awareness of nutrition requirements and the nutritional quality of foods. Indeed, the utilization of new technology often conserves or enhances nutrient content of foods, as well as increasing production and availability. It is worthy of noting also that the proper use of chemicals in food production and processing has *not* resulted in chronic

injury of the consumer—an anxiety expressed by many.

The economic impact of technologic development is of great concern to governmental planners. One index of the economic impact is the expenditure for food expressed as percent of income. In a cash society this index reflects the relatively greater riches—riches available for enhancing the quality of life. In highly industrialized countries of North America and parts of Western Europe, 16 to 22 percent of income is expended on food. At the same time, nutritional deficiencies are notably milder and less prevalent than in areas with less industrialized production of food where 44 to 80% of income is spent for this commodity. Not only is food *relatively* cheaper in industrially developed countries but often is cheaper in absolute cost. Application of science frequently makes a commodity abundantly available at a cheaper price, as exemplified by the decreased retail cost of both turkey and chicken in the United States within the last two decades.

Improved methods of preservation and distribution erase seasonal limits of availability of perishable foods and remove such foodstuffs from classification as "seasonal delicacies." Quality standardization minimizes variation in taste. The resultant psychologic and sensory reactions may reduce the special appeal of a previously rare or exotic food when it becomes commonplace, a reaction that is sometimes misinterpreted by the consumer as an alteration in nutritive value. Contrariwise, nutritional values may be better preserved by such processing than they are in unprocessed or home-processed foods. The products are more sanitary.

There often is failure on the part of national planners, and especially economists, to recognize the enhancement of the *quality of life* through improved health and economic savings that accrue from technology. But such economic advantages of the future may differ from those of the past. They will be determined in part by

the priorities and standards that society sets for political considerations, for educational opportunities, for health, for environmental considerations—e.g., for waste disposal, for elimination of useful agents that may have some environmental impact—for eliminating want, disease and human wastage and for limiting man's seemingly infinite potential for procreation. With these concepts in mind, let us turn to the World Food Conference.

The World Food Conference

The World Food Conference in Rome was proposed by the U.S. Secretary of State³ and called by the Director-General of FAO. Preceding the Conference there was much work on the part of Preparatory Committees, a series of reports on each agenda item, including an "Assessment of the World Food Situation"⁴ and "Proposals for National and International Action Relative to the World Food Problem."⁵

The "Rome Forum"

The day preceding the opening of the Conference, a group of 25 public figures from 16 countries convened under the Chairmanship of Lady Barbara Ward Jackson at the call of the Secretary-General of the Conference.

This "Rome Forum" noted:⁶

World food output has sharply declined;

Grain stocks have fallen and prices have trebled in an uncontrollable market;

There is a sharply increased use of grain for animal products consumed in affluent societies;

It is on the poor with annual per capita income of less than \$200 that the impact falls most grievously.

The Forum urged:⁶

Establishment of an early warning system to predict shortfalls, malnutrition, etc.;

A special investment effort in agriculture of \$18-20 billion per year;

That "old" and "new" rich (OPEC) countries join with developing peoples to agree

upon accelerating investment programs in agriculture, bringing together wealth and managerial skills of developed countries, the investment funds of the wealthiest oil producers and the needs and potential of the poorest countries.

The Forum emphasized:⁶

That the chief hope for sustained and reliable food supply lies in maximum self development of food production by developing peoples.

The Forum ranked:

As first priority to ensure that the benefits of modern agricultural technology—improved seed, fertilizer, water supplies, appropriate use of machinery, better storage and marketing facilities—are extended to the whole farming community and not engrossed simply by those farmers who have the resources to employ the new means or who enjoy access to areas of assured irrigation.

As second priority to integrate a new environmental dimension into farm practices. Agricultural investment therefore should be designed to fit within a rational pattern of land use directed to these purposes.

And as third priority to accompany increased agricultural investment with a really large devotion of new resources to research into the nature of local eco-systems, the possibilities of greater conservation and recycling, new concepts of "eco-development," the scientific improvement of local plants, root crops and animals.

The Forum recognized that variability of traditional agricultural systems, as well as the new possibilities opening up in agricultural and nutritional research, stress the degree to which *education is fundamental to progress and reform to the field of agriculture*. It also requires the development of indigenous scholars and technicians in sufficient numbers to maintain the research and technical development suited to their own country's unique needs.⁶

Hence, a further priority was underscored:⁶

To combine expanded agricultural and educational investment with a wider context

of modernization—in transport and communications, in new settlements, in decentralized industry, in health services, including local centers for family welfare, and in planning new determination to mobilize the small farm sector.

It emphasized⁶ that:

A first priority is to extend the benefits of already available knowledge to the entire farming community.

The World Food Conference and Politics

Dr. Samuel E. Stumpf, Senior Scholar of the Nutrition Foundation, writes of his impressions of the World Food Conference:⁷

Whenever a group of people as diverse as the 2300 delegates from 123 nations come together as they did in November at the World Food Conference in Rome, the discussion inevitably appears complex, confused and contradictory. Nevertheless, it is possible to grasp from the near chaos of the proceedings a definite line of thought...

The impulse to convene the Conference came from the spectre of starvation, imminent and growing in breadth and intensity...

The fact of rapidly growing populations all out of proportion to local food supplies was cited as a major cause of the crisis. The poverty of these populations was a further consequence of their growing numbers. There could be no solution unless a large reduction in population could be achieved. But here disagreement developed. Some argued that controlling the birth rate was a form of personal matter, that this was not an acceptable course according to certain religious values, or, as in case of Brazil, that to increase the size of its population is a "natural right" and is, moreover, necessary if Brazil is to open presently undeveloped parts of its land...

Nothing would seem more simple and straightforward than to mobilize food supplies and send them to the stricken areas. The humanitarian impulse of the "haves" was expressed in commitments of food to the "have nots" even before the Conference was assembled. However, serious questions soon arose: was there enough food for everyone? Who would contribute the food? Who would pay the farmers? Was the era of huge

food surpluses over? Would the food reach the intended targets?...

Many concluded that only through an increase in world food production within the stricken countries, could the growing crisis be managed. Only a program of intensified local food production could possibly overcome the inherent limitations of outside help. Criticism was leveled at suffering nations whose values were said to be misplaced by giving priority to industrial development at the expense of agriculture, the most dramatic case being a program by India to develop an atomic bomb, thereby deflecting efforts to solve the problem of its food supply...

Not only the quantity of food but the need for the nutritionally most useful foods was stressed...

The conference struggled to find a way to achieve the desired objectives of preventing starvation, feeding the hungry and providing proper nutrition. In the debates, one could sense the cross currents of views and emotions generated by a series of charges and counter-charges... It became obvious again that the most promising and indeed indispensable route would be to increase the production of the world food supply.

How can a poor nation break the bonds of poverty and develop a momentum that will breathe life into a stagnant economy? The key word at the Conference was "incentive." What incentives are available to the underdeveloped peoples? Secretary Butz listed the American farmer's incentives as profit, desire to own his own home, educate his children, and pride... The block of underdeveloped nations banded together to fashion a policy which in their judgment would provide the stimulus for a heightened effort on their part to increase their agriculture...

The policy they proposed to the Conference would assure them trade preferences in the markets of developed countries at pegged international commodity prices. Also, they called for the indexing of the prices of their exports to overcome the effects of Western inflation. Should the developing countries aspire to increase their food production by adopting Western style methods? Some argued that to move in this direction would require accepting other Western values which they did not wish to adopt. An alternative was offered by China

where a decentralized communal system of production provides food for its vast population with relatively little dependence upon food imports.

But the Chinese method exacts its own price, particularly in the realm of freedom.

To achieve the level of production required to solve the world food problem assumes that resources would be available to the developing countries, such resources as technical personnel, sources of energy, fertilizers, insecticides and other means for implementing the formulae of the green revolution. Above all, there is a need for investment capital, financial support for this program. The Conference clearly highlighted a new fact in the world, which is the enormous shift of wealth, from the industrial nations to the oil producing powers . . . Even Cuba's Dr. Rodriguez said in his plenary speech that "oil grants rights . . . but also it bestows responsibilities . . ." These concerns generated the most characteristic feature of the Conference, namely, the intense political activity of the contending parties.

Clearly, "politics" was the name of the game at the Conference . . . One nation after another accused the United States, along with a few other large countries of being virtually responsible for the plight of the stricken nations. A typical form of criticism came from the Chinese delegate who charged that the "difficulties of the developing countries were due historically to the plunder and control by colonialism and imperialism of the super powers who had forced a one sided single-product economy and extorted super profits". The "Group of 77," composed of 77 small, new, emerging and underdeveloped countries ("our group has all experienced exploitation of one sort or another") became an unofficial coalition seeking to influence the actions of the Conference. Another considerable contribution was made by a large non-voting group called NGO's (non-governmental organizations). They provided a daily platform for organizations from all parts of the world with an interest and special competence in the world food problem . . .

The political posture of the Conference was evident from the first day when a scramble for the offices of 17 vice presidents and even an associate "rapporteur" produced a hotly contested campaign and an embarrassing delay . . .

Pressure was exerted for the United States to send additional emergency food supplies. Within the U.S. group there were differences on this matter, with Secretary Butz feeling that sufficient commitments by the United States were already in the works and that others should also come forward. At the urging of Senators Hubert Humphrey, George McGovern and Dick Clark, a cable was sent by Secretary Butz asking President Ford to provide these additional supplies, a request the President rejected . . .

In spite of the grim subject, the proceedings produced some humor, as when an early draft of a document urged the nations to "sustain expansion of food production and to improve the consumption of undernourished people.

Results of the Conference

Mr. J. E. Lonning, Chief Executive Officer of Kellogg Company, and a member of the U.S. Delegation states:⁸

The World Food Conference must be viewed as a success in that it represented an opening dialogue. Without such collective action future solutions to the problem of world hunger would not be possible.

This occurred, he notes, in spite of the surprising and frustrating involvement of international politics in such a basic humanitarian endeavor as trying to find ways to feed starving people . . . politics that slowed, and at times stymied, progress on issues. Heartening is his praise of the professional caliber and selfless dedication of all of the government employees in the U.S. delegation, and his comment that "we can take heart that, in some areas at least, we are well served by the employees of the government."

The U. S. position set forth by Secretary Kissinger³ (in his opening address) is based on maximum food production with farmer incentives as its cornerstone. In defining the U.S. position, it was obviously important to limit commitments to what we can realistically do and then do everything possible to fulfill our promises . . . keeping in proper perspective our international role and national goals.

Mr. Lonning⁸ realistically notes that the U.S. involvements should be "within a do-

able time frame, perhaps as much as a quarter of a century, as Secretary Kissinger suggested in his keynote address to the conference. This, of course, means nothing to the millions of people who are starving right now, so we must do all that we can to help these people immediately. But, we must realize that with all the heart in the world we cannot solve world hunger overnight, nor can we solve it by ourselves."

Immediately following the Conference, the U.S. Department of Agriculture summarized⁹ actions of "The World Food Conference in Brief":

Food Production:

The Conference agreed that increased food production is essential in both developed and developing areas. In the case of many developing countries, a reordering of programs, priorities, and farmer incentives is required to stimulate production. The Conference also recognized that additional funds will be required to help developing countries increase production. In this connection, the United States has supported the creation of a voluntary fund. In Rome, the OPEC countries recommended establishment of such a fund. General approval was expressed at the Conference, and follow-up work by the UN will include this proposal. No specific dollar amounts were proposed. In addition, a number of resolutions were passed to improve nutrition programs, child feeding, fertilizer development, and to increase the participation of women in solving world food problems.

Food Aid:

The Conference recommended that food aid donor countries make all efforts, beginning in 1975, to provide commodities and/or financial assistance to ensure at least 10 million tons of grain per year as food aid. The Conference recommended that grain exporting and importing countries, as well as present and potential financial contributors meet as soon as possible on immediate food problems . . .

World Food Security:

The Conference endorsed the FAO undertaking for international cooperation in establishing a world network of national

grain reserves. This would involve adoption of general guidelines for national stockholding policies for grains and the use of international consultations and exchange of information. The Conference gave strong endorsement to the proposal that cereal producing, consuming, and trading nations join together to accelerate implementation of such a world reserve system . . .

Information:

The Conference decided to establish a Global Information and Early Warning System on Food and Agriculture, described by Secretary Butz in his Conference speech as "essential to the whole objective of improved food security around the world." The Conference agreed that FAO is the most appropriate organization to supervise this system. All governments were invited to participate. In the beginning, the system will concentrate on basic foods, particularly grains. Later, a wide range of commodities will be included.

Trade:

The Conference stressed the need for eliminating trade barriers, utilizing the Multilateral Trade Negotiations under the General Agreement on Tariffs and Trade (GATT), as agreed to in the Tokyo Declaration.

World Food Council:

The Conference approved establishment of a World Food Council, an organization to have coordinating, consultative and advisory powers with respect to food aid, investment, and other foreign assistance . . .

The Provisional Report of the Conference¹⁰ contains a number of important recommendations concerning policies and programs to improve nutrition (Resolution V). Among these are recommendations that:

Governments include nutrition education in the curricula for educational programmes at all levels and that all concerned in the fields of agriculture, health and general education be appropriately trained to enable them to further the nutrition education of the public within their domains.

Governments should explore the desirability and feasibility of meeting nutrient deficiencies through fortification of staples

or other widely consumed foods, with amino acids, protein concentrates, vitamins and minerals, and that, with the assistance of WHO in cooperation with other organizations concerned, should establish a worldwide control programme aimed at substantially reducing deficiencies of vitamin A, iodine, iron/folate, vitamin D, riboflavine, and thiamine as quickly as possible.

FAO in association with other international and non-governmental organizations concerned, undertake an inventory of vegetable food resources other than cereals, such as roots, tubers, legumes, vegetables and fruits, including also those from unconventional sources, and that it studies the possibility of increasing their production and consumption, particularly in countries where malnutrition prevails.

Governments take action to strengthen and modernize consumer education services, food legislation and food control programmes and the relevant aspects of marketing practices, aiming at the protection of the consumer (avoiding false and misleading information from mass media and commercial fraud), and that they increase their support of the Codex Alimentarius Commission.

That the joint FAO/WHO food contamination monitoring programme, in cooperation with UNEP, be further developed in order to provide early information to the national authorities for appropriate action.

That a global nutrition surveillance system be established by FAO, WHO, and UNICEF to monitor the food and nutrition conditions of the disadvantaged groups of the population at risk, and to provide a method of rapid and permanent assessment of all factors which influence food consumption patterns and nutritional status.

That governments consider establishing facilities and funds for applied nutrition research related to economic, cultural, social and medical aspects of production, processing, preservation, storage, distribution and utilization of food and that FAO, WHO and UNICEF arrange for an internationally coordinated programme in applied nutritional research including establishing priorities, identifying appropriate research centers and generating the necessary fundings.

That governments should associate, wherever practicable, non-governmental

organizations whose programmes include nutrition-related activities, with their nutritional efforts, particularly in the areas of food and nutrition programmes, nutrition education and feeding programmes for the most vulnerable groups.

Toward these objectives The Nutrition Foundation, in consort with colleagues in Sweden and Britain, has prepared and is publishing¹¹ "Proposed Nutritional Guidelines for the Use of Industrially Produced Nutrients." Guidelines, internationally applicable, will aid in the proper development and adoption of such technologic innovations as called for by the proceeding recommendation. Recognition of common principles and guidelines can promote international agreement and minimize trade restrictions.

Furthermore, The Nutrition Foundation is cooperating with WHO, USAID, and The American Foundation for the Overseas Blind in finalizing a document on control of avitaminosis A and xerophthalmia,¹² a report which stems from a meeting held at Djakarta in December, 1974, and is preparing and distributing appropriate educational material on this subject for health workers. UNICEF and AFOB are assisting with the latter.

Such collaborative associations of governmental and non-governmental organizations can facilitate attainment of the goals of the World Food Conference.

I believe that the thesis of this new dialogue of nations opens an era of widespread realistic assessment of the dimensions of the World Food Crisis and a beginning reapportionment of the responsibilities for measures required to solve or adjust to the problems. The Conference has initiated communication between politicians, planners, economists, nutritionists, and others which can expect to continue. It is a step toward recognizing the "nutrition realities in the lower income countries" called for by Lyle Schertz¹³ who described the greatest reality as "simply the lack of communication between nutritionists and economists" . . . to which list I would add politicians and planners.

The Trust of the Future

As to the future outlook, I support the view so well stated by Dr. George Harrar:¹⁴

Relief is not the ultimate reply to human distress although there have been and will continue to be times and circumstances within which programs for the temporary relief of human misery will be essential. The trust of the future however should be the growing application of knowledge and technology to social and economic problems in order to satisfy universal human needs and minimize the negative aspects of the human experience. Success in this endeavor mandates collective action in which governments and private and public institutions join forces in the attack on those problems which are the most critical retardants to social progress. In the first instance, national governments must evaluate their economies and identify their priorities and the programs which with adequate support can best bring about constructive development . . .

Recognizing the constructive efforts already in progress in the private sector, for example the work of the Agribusiness Council, Dr. Harrar calls for growing interaction between science and business. He emphasizes that: "private agribusiness cannot act in isolation without the cooperation of government; rather it is essential today for both sectors to work in intimate association if world food needs are to be met."

And further highlighting crucial needs for transfer of agricultural technology to underfed, under-productive regions, needs repeatedly identified by conferees in Rome, he concludes:

An important constant in the food energy complex is the scarcity of fertilizer; a key ingredient to the success of the Green Revolution. Agribusiness and science have counseled for years the necessity for major investments in fertilizer production facilities, yet the demand today far exceeds the supply. Growing production and a more equitable distribution of chemical fertilizers is imperative if improvement toward agricultural independence is to occur.

There is growing agreement that these countries must make an all-out effort towards self-sufficiency. This will involve national determination, efficient production systems, the training of personnel, utilization of existing knowledge, and the development of new technologies and the infrastructures which assure optimum production, crop protection, adequate transportation, appropriate market facilities and prices, and credit.

The time has passed when countries can direct meager resources away from the land with the expectation that grain surpluses from the United States, Canada, and Australia will continue to be available. The hope now rests in improving production and in utilizing the extensive collection of information, the available vast and flexible technology, the improved biological materials and a growing body of qualified individuals who are competent in the formulation and implementation of national agricultural development plans. The needs have been delineated; now the commitment must be made.

Private enterprise has perhaps never before faced a more important test of its capacity to deliver in the face of incredible problems.

The penalty of failure is a spectre of want, of need and starvation of inconceivably disastrous proportions. The rewards of success are freedom from hunger and realization of world development through technologic application of scientific knowledge . . . realization of those goals of science so clearly envisioned by Francis Bacon in the 17th century:

. . . the real and legitimate goal of the sciences is the endowment of human life with new inventions and riches . . . not to make imperfect man perfect but to make imperfect man comfortable, happy and healthy.

-
1. W. R. Aykroyd in *The Conquest of Famine*. Pp. 216. Reader's Digest Press, New York, 1975
 2. J. G. Harrar: Nutrition and Numbers in the Third World. *Nutrition Reviews* 32: 97-104, 1974

3. Address by H. A. Kissinger before the World Food Conference: The Global Community and the Struggle Against Famine. Bureau of Public Affairs, Office of Media Services, Washington, D. C., November 5, 1974
4. Preparatory Committee of the World Food Conference: Assessment of the World Food Situation Present and Future. E/CONF. 65/3, November 5-16, 1974
5. Preparatory Committee of the World Food Conference: The World Food Problem Proposals for National and International Action. E/CONF. 65/4, November 6, 1974
6. United Nations World Food Conference: Declaration of the Rome Forum on World Food Problems. E/CONF. 65/14, November 6, 1974
7. S. E. Stumpf: Food, Politics and Survival. *The Reporter* Vol. 6, No. 1, Vanderbilt Law School, Summer, 1975 (in press)
8. J. E. Lonning, personal communication
9. U. S. Department of Agriculture: The World Food Conference in Brief. Fact sheet handed out by Secretary of Agriculture Earl L. Butz at press conference, Washington, D. C., November 18, 1974
10. United Nations Provisional Report E/5587. World Food Conference, November 22, 1974
11. L. Hambræus and W. J. Darby: Proposed Nutritional Guidelines for Utilization of Industrially Produced Nutrients, *Näringsforskning* (in press)
12. Report of a joint WHO/USAID meeting: Vitamin A Deficiency in Xerophthalmia: Priorities for Research Induction Programmes. (in preparation)
13. L. P. Schertz: Nutrition Realities in the Lower Income Countries. *Nutrition Reviews* 31: 201-206, 1973
14. J. G. Harrar: World Food: The Challenge to Agri-Business. (in press)

Nutrition Reviews

authoritative articles by distinguished international scientists • competent, critical, and knowledgeable reviews • interpretative summaries of important original clinical and experimental research • selected excerpts from classic publications marking milestones in nutrition research • informative special reports on government and organization actions • contributing editors review journals of widespread interest covering biochemistry, food science, public health, toxicology, dentistry, pediatrics, internal medicine, gastroenterology and other clinical specialties •

Send subscription and renewal requests to:

THE NUTRITION FOUNDATION
888 Seventeenth Street, N.W.
Washington, D. C. 20006

\$12.00 per year

\$6.00 for students

HEIGHTS AND WEIGHTS OF CHILDREN IN SOUTHERN TUNISIA

11 NOV 1975

Children from the ages of six to 11 years in Southern Tunisia were found to have lower weights and heights than American children of corresponding ages. The growth retardation is mainly due to environmental constraints. The interpretation implicating genetic attributes is questioned.

Key Words: height, weight, Southern Tunisia, socioeconomic status

One of the physiological parameters in children which shows tangible alterations during undernutrition and also after nutritional rehabilitation, is growth. The growth status of children is also generally accepted as a good index of the nutritional status of a community. As such, availability of norms with which the growth rate of a group can be compared acquires considerable importance. Generally, data available from the United States or from a similarly well-developed nation, have been used for comparison. Such comparisons, however, have been criticized because they did not take into account racial and genetic factors. Therefore, lately there have been serious attempts in various countries to establish norms for heights and weights for their own children.

Recently, Lowenstein and O'Connell¹ measured the heights and weights of 205 boys between the ages of six and 11 years in six villages in Southern Tunisia. The data have been compared with those of children from the U.S., Egypt and India. No reasons have been given as to why the last two countries were chosen. One may venture to guess, however, that Egypt had been chosen because of its agroclimatic and possible racial similarities with Southern Tunisia. India, on the other hand, is another developing country with many nutritional problems where substantial data are available for comparison.

The study reveals the obvious, namely the three developing countries lag behind

the U.S., both in height and weight of children. Furthermore, the workers observed that children from India have lower heights and weights than do their counterparts from the two North African countries. The authors chose not to comment on the probable reasons for this. Before considering this to be a true difference, it is well to remember one of the major drawbacks of such surveys in the developing world. In most of these countries the assessment of the exact age of the child is a difficult task.² The authors have not mentioned how the ages of their subjects were assessed. Differences and difficulties in age assessment could have partly contributed to these apparent differences.

Another point open to question in the study is the one pertaining to the children from high socioeconomic groups. They also had heights and weights lower than the American children. The authors argue that these differences may be ascribed to true genetic attributes. There are no well-defined yardsticks by which socioeconomic classes can be defined. The grading has to take not only the per capita income into account, but also the cost of living in the country and the value of the local currency. While the high socioeconomic classes may no doubt be better off than the lower ones in the same country, they may not necessarily be comparable to the U.S. group used as the reference. Studies from India³ showed, on the other hand, that the growth rate of children from the privileged classes is very similar to that of American children. Although what is true of one

country need not be so of another, caution is needed in the interpretation of such observations. By ascribing the differences to genetic and racial factors, programs designed to improve the nutritional status may unfortunately be denied to these children.

The measurements of children belonging to the high socioeconomic group were in between those of the American and lower socioeconomic groups. The differences between children of the two socioeconomic classes could be attributed only to environmental factors. It is now recognized that environmental factors are stronger determinants of growth than are genetic attributes. Of the environmental factors, nutrition is the single most important determinant. The authors consider protein deficiency starting in infancy to be the major factor in the growth retardation of children in Southern Tunisia. Recent studies indicate that calorie inadequacy is the main nutritional constraint on the growth of children in the developing countries.^{4,5} Although one need not swing the pendulum to the other extreme and decry the undue emphasis on

protein deficiency,⁶ it is necessary that cognizance be taken of current concepts. Thus, it is disturbing to note that while the authors have discussed protein deficiency including an exotic subject such as zinc deficiency, they have completely ignored the question of calories. □

1. F. W. Lowenstein and D. E. O'Connell: Selected Body Measurements in Boys Ages 6-11 Years from Six Villages in Southern Tunisia: An International Comparison. *Human Biol.* 46: 471-482, 1974
2. D. B. Jelliffe in *Assessment of the Nutritional Status of the Community*. Pp. 58-63. World Health Organization, Geneva, 1966
3. K. Vijaya Raghavan, D. Singh and M. C. Swaminathan: Heights and Weights of Well-Nourished Indian School Children. *Indian J. Med. Res.* 59: 648-654, 1971
4. D. S. Miller and P. R. Payne: Assessment of Protein Requirements by Nitrogen Balance. *Proc. Nutrition Soc.* 28: 225-234, 1969
5. P. V. Sukhatme: Size and Nature of the Protein Gap. *Nutrition Reviews* 28: 223-226, 1970
6. D. S. McLaren: The Great Protein Fiasco. *Lancet* II: 93-96, 1974

THE INFLUENCE OF DIETARY FAT ON THE COMPOSITION OF THE BODY FAT OF INFANTS

Comparison of the fatty acid composition of the body fat of British and Dutch infants showed that the Dutch infants had a more unsaturated body fat.

Key Words: body fat, infants, milk formula, unsaturated fatty acids

In nonruminant animals the composition of the body fat is profoundly influenced by the type of fat in the diet. A relationship between the composition of dietary fat and the fatty acid composition of adipose tissue has been demonstrated in man¹ although the adipose tissue in adults changes slowly in response to changes in the diet.² In young animals where adipose tissue is being actively laid down very rapid changes are found.³

Widdowson et al.⁴ recently described some observations which show that in the

young infant the composition of the body fat is profoundly influenced by the type of fat being fed to the infant.

In 1973 a study of the composition of a range of infant formulas on sale in Europe showed that one proprietary formula, which was very popular in the Netherlands, had an unusual composition.⁵ In the preparation in question the fat was maize oil with its characteristically high content of linoleic acid and other polyunsaturated fatty acids. In this preparation the linoleic acid provided over half of the total fatty acids compared with 2 percent in a cows' milk formula and about 9 percent in breast milk.

Samples of adipose tissue were obtained from 41 British and 37 Dutch infants. The tissue was obtained at surgery or by needle biopsy from most of the British subjects. In addition, adipose tissue from three anatomical sites was taken from 15 infants who had died. The fatty acid composition of the fats from the different sites was similar and the results from the three sites were combined. The samples from the Dutch infants were all taken by needle biopsy. The ages of the infants providing the samples of adipose tissue ranged from birth to one year of age.

Adipose tissue samples were taken at birth from 14 British and 12 Dutch infants. Between birth and six months of age, 20 British and 19 Dutch infants provided the samples. Two of the British infants in this group were breast fed; the remaining infants were receiving a cows' milk formula. The Dutch infants all received the proprietary formula containing maize oil. Seven British infants were sampled between six and 12 months of age when they were having mixed food, whereas two of the six Dutch infants in this age group were still being fed the formula.

The fat from the adipose tissue samples was extracted and its fatty acid composition measured by gas liquid chromatography. Blood was also taken from the Dutch babies for cholesterol determinations.

The percent of linoleic acid in the fat from the Dutch babies rose rapidly during the first few weeks of life. After two weeks the percent was between 10 and 20, by four weeks it was 25 percent and by 16 weeks it was 32 to 37 percent. In the two older infants, still being fed the formula, the percent of linoleic acid was over 40.

British infants rarely had 3 percent of the body fat as linoleic and many more had less than 1 percent. The proportion of linoleic acid in the fat of the breast fed infants was higher than in the infants fed cows' milk formula. The rise in the proportion of linoleic was accompanied by a fall in the proportions of some other fatty acids, notably myristic and stearic. Palmitic

acid tended to fall as a proportion of the fat after birth. In the infants fed the cows' milk formula the proportions of myristic and stearic acids tended to rise.

Possibly one of the most interesting observations was that the fatty acid compositions of the body fat in the British and Dutch infants differed at birth. The fat of the Dutch infants was appreciably more unsaturated with significantly higher proportions of palmitoleic and linoleic acids. When the body fats of the older infants receiving a mixed diet were compared there still were differences. The fat of the Dutch infants was richer in palmitoleic and linoleic acids.

The results from the newborn infants suggest that maternal dietary fat affects the fetus. This may be a result of the trend in food consumption patterns in the Netherlands which over the past five to ten years showed an increased consumption of margarines with a high polyunsaturated fat content, at the expense of butter. Fatty acids are known to cross the placenta in small amounts.⁶ Therefore, the differences seen in the newborn infants may reflect this change.

As the authors say, it is still a matter of conjecture about the maternal dietary fat affecting the fetal fat. The results show very clearly, however, that substituting the cows' milk fat with maize oil in an infant formula has a profound effect on the body fat of the infants.

A number of questions are raised by this and similar studies which may have important implications and require further work. Does the composition of body fat matter? The Dutch infants tend to have lower serum cholesterol values and this may be an advantage. It is possible, however, that the level of unsaturation in the fat may affect cell multiplication in the adipose tissue.⁷ It is also not clear how extensive the changes in the level of unsaturation of other lipids, the membrane phospholipids, for example, may be. Changes in the composition of such lipids might be expected to have considerable functional significance. The implications of

a highly unsaturated body fat for vitamin E requirements are also far from clear.

This paper demonstrates the pronounced effects on body composition that may result from manipulation of food composition. Rats fed diets containing 20 percent of safflower oil develop adipose tissue containing 60 percent of the total lipid as linoleic acid⁸ and the infant may be nearly as responsive as the rat. Obviously the infant is particularly vulnerable to such changes since one food forms the major part of the diet for a considerable period of time. There is need for a concerted effort to establish a clearer understanding of the significance of such effects. □

1. D. M. Hegsted, C. W. Jack and F. J. Stare: The Composition of Adipose Tissue from Several Parts of the World. *Am. J. Clin. Nutrition* 10: 11-18, 1962
2. J. Hirsch, J. W. Farquhar, E. H. Ahrens, Jr., M. L. Peterson and W. Stoffel: Studies on

Adipose Tissue in Man. *Am. J. Clin. Nutrition* 8: 499-511, 1960

3. J. H. Forsythe, J. Karmarkar and D. M. Hegsted: Deposition and Mobilization of Fatty Acids in Adipose Tissue. *Metabolism* 17: 502-514, 1968
4. E. M. Widdowson, M. J. Dauncey, D. M. T. Gairdner, J. H. P. Jonxis and M. Pelikan-Filipova: Body Fat of British and Dutch Infants. *Brit. Med. J.* 1: 653-655, 1975
5. E. M. Widdowson in *Nutritional Problems in a Changing World*. D. Hollingsworth and M. Russell, Editors, pp. 101-113. Applied Science Publishers Ltd., London, 1973
6. A. J. Szabo, R. D. Grimaldi, and W. F. Jung: Palmitate Transport Across Perfused Human Placenta. *Metabolism* 18: 406-415, 1969
7. M. Launay, M. Richard, R. Alavione and R. Raulin: Excess of Linoleic Acid and Incorporation of Radioactive Precursors on DNA and RNA of Adipose Cells. *Nutrition Rept. Int.* 5: 339-348, 1972
8. J. H. Forsythe, R. Shaftel and D. M. Hegsted: Deposition of Linoleic and Linolenic Acid in Rat Adipose Tissue. *J. Nutrition* 96: 157-162, 1968

ABSORPTION OF EXTRINSIC AND INTRINSIC IRON LABELS

Recent data demonstrate that a two-pool concept of iron absorption is valid and can be used to measure the absorption of the biological iron mixed diets by use of extrinsic labeling of the dietary iron.

Key Words: iron absorption, humans

The use of a variety of foodstuffs labeled biologically with radioiron as a technique for measuring iron absorption has been investigated since 1951.¹ In most of the subsequent studies only one labeled food was studied at a time. These studies revealed clearly that other dietary constituents as well as physiological state of the subject could influence iron absorption but could not provide reliable data on the absorption of iron from a complete diet whose iron was derived from a variety of sources.

In recent years double isotope techniques have been employed to investigate the absorption of iron from biologically labeled foods relative to iron mixed into

the food at the time of preparation. Björn-Rasmussen and his co-workers² used ⁵⁹Fe intrinsically labeled corn and eggs mixed with extrinsic ⁵⁵FeCl₃ and ⁵⁵Fe intrinsically labeled wheat with extrinsic ⁵⁹FeCl₃ in separate studies with healthy young men. Radioiron absorption was calculated from the measurement of blood radioactivity and an estimate of blood volume two weeks after administering the isotopes. In each case they found the ratio of extrinsic to intrinsic labeled iron absorption not to differ from unity and concluded that there was a complete isotopic exchange between the biological label and the added tracer.

This technique has also been successfully investigated by Layrisse and Martinez-

Torres with reference to absorption of heme iron.³ This work was undertaken in view of the concept that absorbable iron can be viewed as consisting of two pools, non-heme and heme iron. Veal muscle was intrinsically tagged by injecting a calf with ^{59}Fe and obtaining the muscle three months later. The extrinsic tag was hemoglobin isolated from the blood of a rabbit that had been treated previously with ^{55}Fe . They also used intrinsically labeled corn and incorporated an extrinsic label of inorganic iron in the same manner as the preceding authors. The subjects were 94 Venezuelan peasants exhibiting a wide range of iron states which provided a wide range of iron absorption. The iron sources were incorporated in a mixed meal of black beans, corn, rice and veal. They found that the heme iron of hemoglobin from rabbit blood was absorbed at the same rate as the veal iron, which was 80 percent heme iron and 20 percent ferritin, and that the inorganic iron was absorbed at the same rate as the non-heme iron of corn and at a lower rate than the iron of veal.

On the basis of these and other studies which demonstrated the validity of the extrinsic tag two-pool method, Björn-Rasmussen and his co-workers⁴ made a more detailed study. This involved the examination of iron absorption from separate meals, of the increase in iron absorption obtained by iron fortification and of the practical applicability of this technique for measuring iron absorption from the whole diet.

A total of four studies was conducted. Extrinsic labels were used for one day in the first three experiments and for two days in the fourth. In study I the total daily absorption of both heme and non-heme iron was measured. $^{55}\text{FeCl}_3$ and ^{59}Fe labeled rabbit hemoglobin were used as extrinsic tags. In the remaining studies only non-heme iron absorption was measured. In study II $^{59}\text{FeCl}_3$ was incorporated in the breakfast and $^{55}\text{FeCl}_3$ in lunch and dinner, while in study III $^{59}\text{FeCl}_3$ was used in lunch only and $^{55}\text{FeCl}_3$ was used in breakfast and dinner,

thus permitting the study of non-heme iron absorption from breakfast or lunch in comparison to the other two meals. In study IV $^{59}\text{FeCl}_3$ was added to all meals and $^{55}\text{FeCl}_3$ was added in the meals fed the second day to permit measurement of non-heme iron absorption in the presence of an additional iron supplement. Total iron intake in all studies was 17.4 mg daily except that in day two of study IV it was 26.5 mg, the increase being in the form of ferrous sulfate. In all cases the heme iron intake was 1.0 mg.

The subjects were 32 young men in their seventh month of military service. The meals were designed to contain the relative amounts of foods representing the average meals served at the military unit. The foods used were thoroughly minced and mixed and the extrinsic labels and iron supplements were added at time of mixing. The mixes were served as cooked puddings.

The absorption of ^{59}Fe was measured in a whole body counter, measurements being made 30 minutes after a ^{59}Fe meal and two weeks later. Relative absorptions of ^{59}Fe and ^{55}Fe were measured from radioactivities of the foods consumed and of the blood sampled two weeks later. Total ^{55}Fe absorption was measured from the relative absorption ratio in the blood and the total ^{59}Fe absorption measured in the whole body counter.

In the study I the non-heme iron absorption was 5.3 ± 1.8 percent of the intake, whereas the heme iron absorption was 37.3 ± 3.8 percent. The total absorption of dietary iron amounted to $1.25 \pm .32$ mg. In study II the non-heme iron absorption from the breakfast meal was 2.1 ± 0.9 percent and that from lunch-dinner was 3.1 ± 0.8 percent. The total non-heme iron absorption was $0.46 \pm .14$ mg in contrast to non-heme iron absorption of 0.88 ± 0.3 mg in study I. In study III non-heme iron absorption from lunch was 4.0 ± 1.1 percent and from the combination of breakfast and dinner was 4.0 ± 1.0 percent. The total non-heme iron absorption was $0.66 \pm .17$ mg. In study IV non-heme iron absorption was 3.5 ± 0.6 percent from all three

meals ($0.57 \pm .10$ mg) when the intake was 16.4 mg. On the second day when the non-heme iron intake was increased to 25.5 mg its absorption was 4.0 ± 0.8 percent ($1.02 \pm .19$ mg).

Although this series of experiments was not designed to test critically the accuracy of the methods employed, the results do seem reasonable. In study II, for example, the difference of 1 percent in non-heme iron absorption between breakfast and dinner-lunch is statistically real when analyzed by the paired "t" test. In other words, in spite of the rather large variation between individuals, their response in absorption between the two measurements was quite uniform. Also, the direction of difference, greater non-heme iron absorption from the lunch-dinner meal, is in agreement with the principle that heme iron, present only in lunch-dinner, improves absorption of non-heme iron.⁵ Also, there was more phytate in the breakfast than in lunch-dinner and this would tend to inhibit iron absorption.

Further evidence that there are no large systematic errors is that the average total iron absorption in all of these studies was 1.02 mg daily, an amount closely approximating estimates of iron excretion in healthy young men.

The work of these two groups of investigators demonstrates the utility of the extrinsic tag method for studying iron absorption in humans. The method obviates the need of using intrinsic labeling of foods, is applicable to relatively large numbers of subjects and is more accurate than conventional balance techniques. It is probably not useful in the testing of specific sources of dietary iron supplements whose solubility would place their absorption outside of the limits of the usual two-pool concept. The method has been validated in a recent study with adult rats.⁶ Non-heme iron absorption in these animals responded to many of the factors known to influence absorption in humans, i.e., anemia, dietary ascorbic acid and presence of heme iron,

although the iron absorption values are too high to be extrapolated to humans.

In all of these studies the extrinsic non-heme label was supplied as the soluble FeCl_3 on the assumption that non-heme food iron would have similar solubility and would be equally affected by luminal factors in the intestine. To the extent that food iron supplements such as relatively insoluble iron phosphates or iron contamination in the form of oxide or other less soluble forms are present, this method would overestimate availability of the total non-heme iron intake. These considerations appear to place limits on the general applicability of both the extrinsic and intrinsic tag methods. Nonetheless the extrinsic tag procedure does appear to be as useful an approach as is currently available for measuring absorption of the biological iron content of the diets. \square

-
1. C. V. Moore and R. Dubach: Observations on the Absorption of Iron from Foods Tagged with Radioiron. *Trans. Assn. Am. Physicians* 64: 245-256, 1951
 2. E. Björn-Rasmussen, L. Hallberg and R. B. Walker: Food Iron Absorption in Man. 1. Isotopic Exchange between Food Iron and Inorganic Iron Salt Added to Food: Studies on Maize, Wheat, and Eggs. *Am. J. Clin. Nutrition* 25: 317-323, 1972
 3. M. Layrisse and C. Martinez-Torres: Model for Measuring Dietary Absorption of Heme Iron: Test with a Complete Meal. *Am. J. Clin. Nutrition* 25: 401-411, 1972
 4. E. Björn-Rasmussen, L. Hallberg, B. Isaksson and B. Arvidsson: Food Iron Absorption in Man. Applications of the Two-Pool Extrinsic Tag Method to Measure Heme and Nonheme Iron Absorption from the Whole Diet. *J. Clin. Invest.* 53: 247-255, 1974
 5. C. Martinez-Torres and M. Layrisse: Iron Absorption from Veal Muscle. *Am. J. Clin. Nutrition* 24: 531-540, 1971
 6. E. R. Monson: Validation of an Extrinsic Iron Label in Monitoring Absorption of Nonheme Food Iron in Normal and Iron-Deficient Rats. *J. Nutrition* 104: 1490-1495, 1974

NUTRITION CLASSICS

THE JOURNAL OF NUTRITION

VOLUME 18

JULY—DECEMBER, 1939

Pages 1 — 13

RETARDED GROWTH, LIFE SPAN, ULTIMATE BODY SIZE AND AGE CHANGES IN THE ALBINO RAT AFTER FEEDING DIETS RESTRICTED IN CALORIES

C. M. McCAY, L. A. MAYNARD, G. SPERLING AND LeROY L. BARNES

Animal Nutrition Laboratory, Cornell University, Ithaca, New York

Diets used in these studies were made very high in percentage of such constituents as protein, minerals and vitamins in order to insure an adequate ingestion of these essentials. The retardation is effected by the deficiency in the daily allowance of energy in the diet. In the present study every animal was given the same daily allowance of the basal diet. Animals permitted to grow normally were given additional calories.

• • •

The amount of this diet fed to individuals daily was determined by the amount needed to maintain the retarded animals at a stationary body weight. The results of such a regime insure the ingestion of

approximately equal amounts of such essentials as protein and minerals, but do not impose any additional burden upon such organs as the kidneys in the case of animals allowed to grow normally. In this case all animals ingested enough protein, vitamins and minerals for growth, but the retarded animals could not grow due to a deficiency of calories.

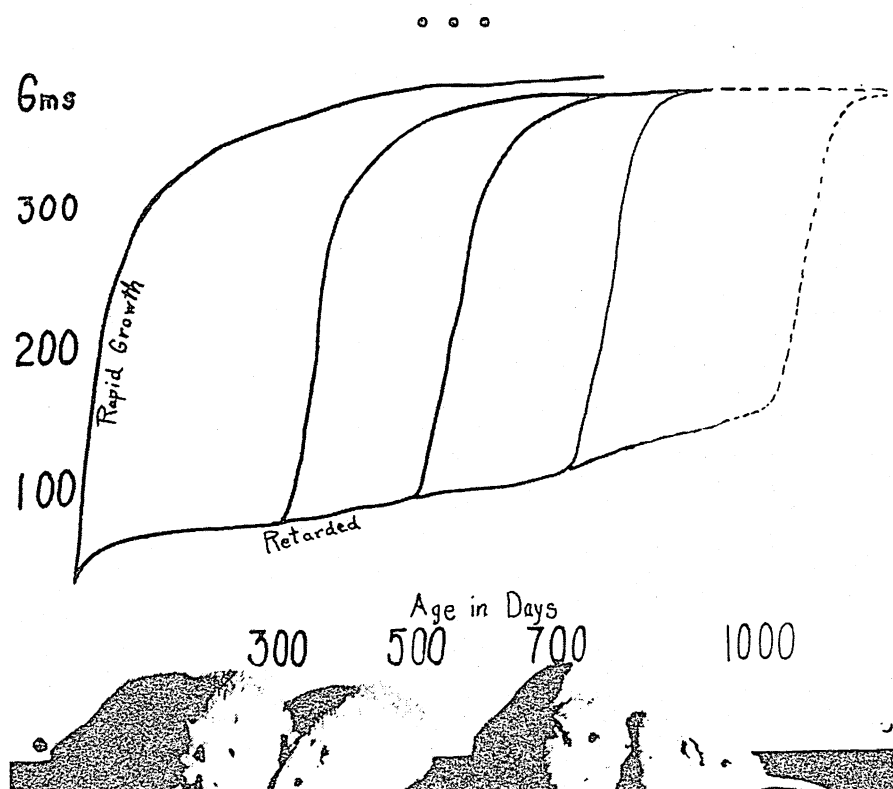


Figure 1. Projected growth curves showing the plan for the experiment and representatives of the retarded groups at the age of 1000 days.

At the end of 300 days the retarded animals were divided into four groups as nearly equal as possible. One was fed additional calories to permit growth. The other groups were thus assigned at this date to the several periods for resuming growth. These were at the end of 500, 700 and 1000 days. The design of the experiment is shown in figure 1.



Figure 2. Age 964 days. This photograph was taken the last day of life for the last of the controls on the left and 36 days before the retarded rat on the right was allowed to mature. Note the contrast in degree of senescence.

• • •

SUMMARY

Rats were retarded in growth for periods of 300, 500, 700 and 1000 days before being allowed to grow to maturity. Members of each of these groups were alive when the last of the control groups had died at an age of 965 days. Retardation of growth by diets, complete except for calories, affords a means of producing very old animals for studying aging. Animals that are retarded for even 300 days can never become as large as those that mature normally. After 1000 days of retardation only part of the rats were able to resume growth when adequate energy was allowed in the diet. Even at this extreme period the male tended to grow to a larger size than the female. The growth of the bones in rats retarded for 700 and 1000 days was followed by means of x-ray photographs. The maintenance of a constant body weight in this period of old age does not check the growth of the bones. These increase slowly and respond to realimentation in all cases after 700 days of retardation but in only part of the cases after 1000 days.

PROTEIN-BOUND RETINOL OR A RETINOL METABOLITE IN URINE

Retinol or a retinol metabolite bound to protein was found in rat and human urine. Its excretion decreased in vitamin A-deficient rats. A prosthetic group with 330 nm absorption or Carr-Price reactivity could not be extracted from the material.

Key Words: retinol, retinol-binding protein, retinol metabolite, urinary excretion, protein-bound retinol

Urinary excretion of water soluble vitamins or their metabolites (e.g. 4-pyridoxic acid) is a useful index of the status of vitamin nutrition in man and animals. A similar approach has not been applied to retinol because retinol, as such, is rarely detected in urine. Information on the metabolism or the metabolic role of this vitamin is also fragmentary.

Retinol bound to the transport protein retinol-binding protein (RBP) has been isolated from the urine of patients suffering from tubular proteinuria due to cadmium poisoning or other renal diseases.¹ In a recent study Clark and colleagues² tried to isolate holo RBP (RBP bound to retinol) from the urine of cadmium-treated rats. They failed to obtain this metabolite despite marked proteinuria. Instead they isolated a protein of small molecular weight, with retinol or a retinol metabolite bound to it.

Retinol-15 ³H was injected intraperitoneally into rats and the urine fractionated on Sephadex G-100, after dialysis and lyophilization. Two protein fractions (peaks A and B) could be detected with ultraviolet absorption at 330 nm. There was a marked increase in protein and the 330 nm absorbing material after CdCl₂ poisoning. In the urine of vitamin A-deficient rats, the concentration of both these metabolites was markedly lower than in normal rat urine.

Peak A which was eluted near the void volume of the Sephadex G-100 column, was at first thought to be a RBP-retinol complex. No 330 nm absorbing material could be extracted from it with ether, nor could retinol be split from it by dialysis. Its molecular weight seemed to be very much higher than that of the RBP-prealbumin complex.³

The major metabolite, peak B, showed only a slight shoulder at 330 nm. Its fluorescence spectrum gave an emission maximum at 426 nm (excitation maximum at 344 nm) with an intensity 12.3 times that of retinol (excitation 337 nm, fluorescence 465 nm), for equivalent absorption at 330 nm. When injected into vitamin A-deficient rats, it failed to cure vitamin A deficiency. No radioactivity, 330 nm absorbing or Carr-Price reactive material could be extracted from it with lipid solvents, or after denaturation of the protein with trichloroacetic acid. The metabolite had sufficient protein to be measured by the Lowry reaction. The molecular weight of the peak B material was determined to be about 4600 by chromatography on Sephadex G-50 column, calibrated with cytochrome c, insulin and bacitracin.

To make sure that the excreted substance was a true metabolite, rather than retinol attached nonspecifically to a urinary protein, labeled retinol was added in vitro to normal unlabeled urine and then examined for the label in peak B. Little radioactivity could be detected, suggesting that the substance was a true metabolite.

From these experiments the authors conclude that the rat excretes a protein complexed, possibly covalently, with retinol or a metabolite of retinol with molecular weight of about 4600. If it is a metabolite, it cannot be retinoic acid since the tritiated atom was in position 15. The shift to a lower wavelength in the fluorescence spectrum also suggests that it may not be retinol.

The absence of a pronounced 330 peak, like that shown by RBP, is surprising since the latter has a larger amount of protein per mole of retinol.⁴ It is possible that the 330 nm absorption in peak B is due to a metabolite of retinol which has lower absorptivity than retinol, or that retinol is attached to a small fragment of RBP.

The large increase in the excretion of the substance after CdCl₂ poisoning suggests that while a small amount is excreted in normal urine, much of it is reabsorbed through the kidney tubules. Interestingly a substance with similar ultraviolet absorption, fluorescence spectrum and molecular

weight characteristics was also identified in normal human urine. It would be worthwhile to see if this metabolite can be an indicator of vitamin A status in man. It is easily isolated from urine, can be identified by its strong fluorescence and at least in rats, its excretion diminishes in vitamin A deficiency. □

-
1. P. A. Peterson and I. Berggård: Isolation and Properties of a Human Retinol-Transporting Protein. *J. Biol. Chem.* 246: 25-33, 1972
 2. J.N. Clark, R.S. Nathaniel, H.K. Kleinman and G. Wolf: Protein-Bound Retinol or a Retinol Metabolite Found in Rat and Human Urine. *Biochem. Biophys. Res. Commun.* 62: 233-240, 1975
 3. P. A. Peterson: Characteristics of a Vitamin A-Transporting Protein Complex Occuring in Human Serum. *J. Biol. Chem.* 246: 34-43, 1971
 4. D. S. Goodman and A. Raz: Extraction and Recombination Studies of the Interaction of Retinol with Human Plasma Retinol-Binding Proteins. *J. Lipid Res.* 13: 338-347, 1972

FTC PROPOSED REGULATIONS ON FOOD ADVERTISING

The January 1975 issue of *Nutrition Reviews* noted the November 11, 1974 publication by the Federal Trade Commission of a Proposed Rule regarding food advertising. Excerpts are presented below (smaller typeface). Some of the issues which the FTC raised are also included (standard typeface). The staff statement in support of the proposed rule, the staff statement in support of affirmative disclosure in food advertising, and the staff proposal on affirmative disclosure are not reprinted here. Copies of the complete publication are available from FTC.

All interested persons may file written data, views or arguments to William D. Dixon, Special Assistant for Rulemaking, Federal Trade Commission, Washington, D.C. 20580. Comments will be accepted until ten days before the Public Hearings.

SUBPART A: GENERAL

437.1 Definitions.

437.2 Form, content and method of making disclosures.

SUBPART B: VOLUNTARY CLAIMS

437.3 Emphatic nutrition claims.

437.4 Nutrient comparison claims.

437.5 Nourishment claims.

437.6 Natural and organic food claims.

437.7 Claims for foods intended to be combined with other foods.

437.8 Energy and calorie claims.

437.9 Fat, fatty acid and cholesterol content claims.

437.10 Health and related claims.

SUBPART A: GENERAL

§ 437.1 DEFINITIONS.

For the purpose of this Rule the following definitions shall apply:

(a) "Advertisement" or "Advertising"

Any written or verbal statement, illustration, or depiction, other than a label or in the labeling, which is designed to effect the sale of any food product, or to create interest in the purchase of such product, whether the same appears in a newspaper, magazine, leaflet, circular, mailer, book insert, catalog, sales promotional material, other periodical literature (except professional or scientific journals), billboard, public transit card, or in a radio or television broadcast or in any other media. . .

(b) "Food"

Any article used for food or drink by humans, including chewing gum. However, it does *not* include:

(1) Special formula foods which are developed, intended or marketed exclusively for infants (persons not more than 12 months of age) and which provide the complete nutritional requirements of infants.

(2) Foods represented for use solely under medical supervision to meet nutritional requirements in specific medical conditions and advertised only in professional journals or publications.

(3) Alcoholic beverages subject to the provisions of the Federal Alcohol Administration Act of 1935 (27 U.S.C. §201 *et seq.*).

(c) "Nutrients"

Protein and those vitamins and minerals listed in 21 CFR §1.17(c) (7) (iv) and 21 CFR §125.1(b).

(d) "United States Recommended Daily Allowances" (U.S. RDA)

The nutrients and levels established, subject to amendment, in 21 CFR §125.1.

The definition specifically includes protein and those other "primary" and "optional" nutrients for which a U.S. Recommended Daily Allowance (U.S. RDA) has been established, namely vitamin A, vitamin D, vitamin E, vitamin C, folic acid, thiamine, riboflavin, niacin, vitamin B₆, vitamin B₁₂, biotin, pantothenic acid, calcium, phosphorus, iodine, iron, magnesium, copper, and zinc.

1. Would it better serve the purposes of the Proposed Rule to expand the definition of "nutrients" to include nutrients for which there is no established U.S. RDA? If so, which nutrient(s)?

(e) "Serving"....

(f) "Portion"....

(g) "Clearly and Conspicuously Disclose"

(1) Disclosing in a manner which can be easily understood (in the case of television and print advertising, also easily seen and read) by the casual observer, listener, or reader among members of the public....

(h) "Representation" or "Represent"

Any direct or indirect statement, suggestion or implication in advertising, including but not limited to one which is made orally, in writing, pictorially, or by any other audio or visual means, or by any combination thereof.

(i) "Protein Efficiency Ratio" (PER)....

§ 437.2 FORM, CONTENT AND METHODS OF MAKING DISCLOSURES.

Any disclosure required or described by any provision of this Rule shall be made in accordance with the following general provisions of this Rule and as may be specifically prescribed in a section dealing with that particular disclosure.

(a) Nutrients

(1) Any advertisement which contains a representation concerning a nutrient or a disclosure of a nutrient shall make such representation or disclosure only from among the nutrients listed in 21 CFR § 1.17(c) (7) (iv) and 21 CFR § 125.1(b).

(2) A food shall not be represented in advertising as containing a nutrient, unless (a) the nutrient's (i) identity (stated as the common or usual name) and (ii) amount (expressed as a percentage of the U.S. RDA contained in a stated serving of the advertised food) are clearly and conspicuously disclosed in accordance with all the provisions of this subpart of this Rule, and unless (b) a serving of such food contains the identified nutrient in an amount of 10 percent or more of the U.S. RDA; *provided, however*, that, in instances where a food or a serving thereof is *not* required to contain a nutrient at a certain percentage of the U.S. RDA before a voluntary claim is made, an advertisement may represent the presence of nutrients contained in amounts of less than 10 percent of the U.S. RDA per serving if the identities of all nutrients required to be disclosed by 21 CFR § 1.17 when a nutrition claim is made, as well as their respective percentages of the U.S. RDA per serving (including zero percent), are clearly and conspicuously disclosed in accordance with all of the provisions of this subpart of this Rule. If a food or a serving thereof is required to contain any nutrient at a certain percentage of the U.S. RDA before a voluntary claim may be made (see Subpart B), the actual percentage (prior to any rounding off) of the U.S. RDA at which any such nutrient is contained in the advertised food or a serving thereof shall determine whether the condition(s) for making the claim has (have) been satisfied.

(3) An advertised food or a serving thereof may not be represented as containing a nutrient in an amount of 50 percent or more of the U.S. RDA, unless such food or serving contains the identified nutrient only in a natural occurring (indigenous) form or such nutrient has been added in compliance with 21 CFR § 1.17(a) (2).

.....

1. Is it appropriate to permit in advertising (television? radio? print?) representations regarding nutrients which are *not* present in nutritionally significant amounts in view of the fact that a disclosure of the complete nutrition profile of specific nutrient data on the label will be required; or should such truthful representations be prohibited in advertising (television? radio? print?) due to the possibly misleading implications of nutritional significance that might fol-

low from any reference to the presence of such nutrients?

(b) Protein

(1) The percentage of the U.S. RDA of protein present per serving of the advertised food shall be based on a U.S. RDA of 45 grams of protein, if the total protein has a PER equal to or greater than the PER of casein; or based on a U.S. RDA of 65 grams of protein, if the total protein has a PER less than that of casein.

(2) Except with respect to the amount of protein as permitted by the proviso in subparagraph (a)(2), above, representations in advertising of the presence of protein may be made only if a serving of the advertised food contains protein at a level of 10 percent or more of the U.S. RDA and the total protein in the advertised food alone has a PER of 20 percent or more of the PER of casein.

1. Are the proposed standards for protein quality and quantity sufficiently high? If not, what other standards might be established which would be sufficiently compatible with the nutrient labeling program standards so as to neither confuse consumers nor unreasonably burden advertisers?
2. Should the proviso contained in subparagraph 437.2(a) (2) apply to representations regarding the quantity of protein as well as such representations relating to those vitamins and minerals considered as "nutrients"?

(c) Analytical Methods. . . .

(d) Calories. . . .

(e) Serving or Portion

Statements regarding servings or portions shall be consistently stated in terms of a convenient unit of such food or a convenient unit of measure that can be easily identified as an average or usual serving or portion and can be readily understood as such by purchasers of such food. . . .

(f) Identification and Designation of Foods. . .

(g) Television Advertisements — Method and Form of Disclosures.

Under paragraphs 437.2 (a) and (b) and Subpart B of this Rule, any disclosure in any

television advertisement shall be made in the same portion (audio or video) of the advertisement in which the voluntary claim is made. The video portion of the disclosure in each such advertisement shall be prominently displayed in the form of a super or title, or prominently displayed on the screen by itself so as to enable it to be completely and easily seen and read on all television sets, regardless of picture tube size, that are commonly available for purchase by the consuming public. Any disclosure required by Subpart B of this Rule in any advertisement shall be made in immediate conjunction with the voluntary claim which creates the requirement for such disclosure.

(h) Print Advertisements — Method and Form of Disclosures

(1) Any disclosure in a print or display advertisement shall be prominently displayed.

. . . .

SUBPART B: VOLUNTARY CLAIMS

§ 437.3 — EMPHATIC NUTRITION CLAIMS.

Emphatic, extraordinary, positive or similar claims concerning the nutritional value of a food with general or specific reference to any nutrient(s) contained in such food, including but not limited to the use of terms such as "lots (or "full) of____," "high (or "rich) in____," "packed (or "loaded) with____," and "excellent (or "significant or "good) source of____" shall not be used in advertising unless:

(a) The identity of any nutrient upon which the claim is based, as well as the percentage of the U.S. RDA per stated serving provided by each such identified nutrient, is clearly and conspicuously disclosed; and

(b) A serving of the advertised food contains each nutrient identified pursuant to paragraph (a) hereinabove in an amount of at least 35 percent of the U.S. RDA.

1. Is the fact that a serving of food contributes 35% of the U.S. RDA of the claimed nutrients a sufficient contribution to the diet to justify an emphatic claim?
2. Is there a lower percentage contribution which is sufficient to justify an emphatic claim?

§ 437.4 NUTRIENT COMPARISON CLAIMS.

(a) Representations in advertising which make a comparative claim for the amount of any nutrient contained in an advertised food shall not be made, unless:

(1) The comparison is with an equal-sized serving of a commercially available food; and

(2) If a serving of the advertised food contains the same number of calories as or fewer calories than an equal-sized serving of the compared food, the compared food contains no more than two nutrients in amounts greater by 10 percent or more of the U.S. RDA than the amounts (including zero percent) at which the same two nutrients are contained in a serving of the advertised food; and

(3) If a serving of the advertised food contains more calories than an equal-sized serving of the compared food, the compared food contains no more than two nutrients in amounts greater on a per 100 calorie basis than the amounts (including zero percent) at which the same two nutrients are contained in a serving of the advertised food; and

(4) If the comparison concerns protein, a serving of the advertised food contains protein of at least the same quality as that contained in an equal-sized serving of the compared food; and

(5) The identities of the advertised and compared foods are clearly and conspicuously disclosed; and

(6) The advertised food and the food with which it is compared normally serve the same purpose in the diet; and

(7) The same nutrients are compared and the name of each such compared nutrient is clearly and conspicuously disclosed; and

(8) The percentage of the U.S. RDA of each compared nutrient provided by a stated serving of the advertised food is clearly and conspicuously disclosed; and

(9) If an advertised food is represented as one which contains any nutrient in any amount greater than the amount of such nutrient in another food, the amount contained in a serving of the advertised food exceeds that contained in an equal-sized serving of the compared food by at least 10 percent of the U.S. RDA.

1. Should nutrient comparison claims permitted by the Proposed Rule be limited to foods which serve the same purpose in the diet?

2. Should the test for permitting a comparison between the quantity of a specific nutrient in the advertised food and the amount of that nutrient in the compared food be based on an overall comparison between those nutrients contained in the compared food and the *same* nutrients in the advertised food; or should the quantities of each nutrient in the compared food be compared with the quantity at which *any* nutrient is contained in the advertised food? Are there additional or alternative tests of nutritional equivalence which should be met before a nutrient comparison claim may be made?

(b) A food shall not be represented in advertising to be a substitute or replacement for another food (unless it is a food labeled "imitation" in compliance with 21 CFR §1.8), or as nutritious as another food, unless:

(1) A serving of the advertised food contains at least the same nutrients as those nutrients contained in an amount of 2 percent or more of the U.S. RDA in an equal-sized serving of the compared food, and each such nutrient is present in the advertised food in an amount which is at least equivalent to the amount at which each is contained in an equal-sized serving of the compared food; and

(2) If the compared food contains protein, a serving of the advertised food contains protein of at least the same quality as that contained in an equal-sized serving of the compared food; and

(3) The identity of the compared food and number of calories provided by equal-sized, stated servings of the advertised and compared foods, respectively, is clearly and conspicuously disclosed; and

(4) If the advertised food contains a higher fat content than the compared food, such fact, as well as the total fat content (in accordance with 21 CFR §1.17(c) (6) and 1.18(c) (2) (i)), is clearly and conspicuously disclosed; and

(5) If an advertisement is for a food labeled "imitation" in compliance with 21 CFR §1.8, it is clearly and conspicuously disclosed that such food is not as nutritious as the food for which it is intended to be a substitute or replacement.

1. Is it appropriate to require a serving of the advertised food to contain *all* the nutrients contained in "measurable amounts" (i.e., 2% of the U.S. RDA) in at least the same amounts as they are contained in a serving of the compared food? If not all nutrients, as to what nutrients should there be parity?
2. Should nutrient comparisons (for purposes of satisfying the first prerequisite of making a claim under paragraph 437.4(b)) be between nutrients contained not at 2 percent but at some higher level (e.g., 10% of the U.S. RDA) in a serving of the compared food?
3. Will disclosures of comparative caloric and fat content be meaningful to consumers in the context of these claims or should there be prerequisites to the making of such claims which require that a serving of the advertised food have as many calories as, or fewer calories than, a serving of the compared food, and that a serving of the advertised food have the same fat content as, or a lower fat content than, a serving of the compared food, without any requirement of disclosure?

(c) A food shall not be represented in advertising to be nutritionally superior to another food, unless:

(1) The nutrients in a serving of the advertised food provide at least 10 percent more of the U.S. RDA than are provided by those nutrients contained in an amount of 2 percent or more of the U.S. RDA in an equal-sized serving of the compared food; and

(2) If the compared food contains protein, a serving of the advertised food contains protein of at least the same quality as that contained in an equal-sized serving of the compared food; and

(3) The identity of the compared food and number of calories provided by equal-sized, stated servings of the advertised and compared foods, respectively, is clearly and conspicuously disclosed; and

(4) If the advertised food contains a higher fat content than the compared food, such fact, as well as the total fat content (in accordance with 21 CFR § 1.17(c) (6) and

1.18(c) (2) (i)), is clearly and conspicuously disclosed.

1. Is it appropriate to require a serving of the advertised food to contain all the nutrients contained in "measurable amounts" (i.e., 2% of the U.S. RDA) in a serving of the compared food, in amounts greater by 10% or more of the U.S. RDA than the amounts contained in the compared food, before a nutritional superiority claim can be made? If not all nutrients, as to which nutrients should a comparison be made?
2. Should nutrient comparisons (for purposes of satisfying the first prerequisite of making a claim under subparagraph (c) (1)) be between nutrients contained not at 2 percent but at some higher level (e.g., 10% of the U.S. RDA) in a serving of the compared food?

§ 437.5 NOURISHMENT CLAIMS.

(a) An advertisement shall not represent a food to be "nourishing," "wholesome," "nutritious," or use any other term of similar import which in any way states, suggests or implies that such food is a valuable or significant source of nutrition, unless a serving of the food contains at least four nutrients, including protein, each of which is present in an amount of at least 10 percent of the U.S. RDA per 100 calories, and unless at least one of such nutrients is present in a serving of such food in an amount of at least 10 percent of the U.S. RDA; *provided, however*, that such terms may be used to describe any identified nutrient(s) which is (are) contained in such food (e.g., "nutritious Vitamin C"), subject to the provisions of subparagraph 437.2(a) (2) of this Rule.

1. Should the prerequisite for making a claim that a food is a valuable or significant source of nutrition be based on the content of 4 nutrients, *including protein*, in amounts of 10% of the U.S. RDA per 100 calories or should it be required that those or some of those 4 nutrients be present in amounts of 10% of the U.S. RDA per serving of the advertised food? Are there additional or alternative requirements that should be met before such a claim is allowed?

(b) A food or a serving thereof shall not be represented in advertising as providing all of the nutrients necessary for a sound, complete or balanced diet, unless it satisfies the U.S. RDA requirements for protein, vitamins and minerals prescribed in 21 CFR Part 125, and unless competent and reliable scientific tests demonstrate that such food is a total diet replacement.

(c) Subject to the provisions of paragraph (b) hereinabove, an advertisement shall not represent that an advertised food or a serving thereof alone is "perfect" or "nutritionally perfect," provides "complete nutrition," contains "all the good things you need," or use any other term of similar import which in any way states, suggests or implies that consumption of only the advertised food will provide enough nutrition to constitute a sufficient and full source of nutrition; or that consumption of the advertised food or a serving thereof maintains health, makes an individual well-fed or in any way is a unique, special or exclusive source of nutrition or health benefits.

(d) An advertisement shall not represent that a food or a serving thereof constitutes a nutritionally adequate meal, unless such advertised food or serving thereof complies with an applicable federal regulation prescribed in the Code of Federal Regulations.

• • • •

*§ 437.6 NATURAL AND ORGANIC FOOD CLAIMS.

(a) A food shall not be represented in advertising to be "natural" or a "natural food" or "naturally grown," or otherwise be depicted, designated or described by any term or demonstration of similar import. However, an advertisement may represent that a food does not contain any artificial or synthetic preservatives, flavors or colors, or any other artificial or synthetic ingredients, if such is the fact.

(b) A food shall not be represented in advertising to be "organic" or an "organic food" or "organically grown," or otherwise be depicted, designated or described by any term or demonstration of similar import. However, an advertisement may represent that a food has not been grown with or subjected to pesticides or artificial fertilizers or artificial conditioners, if such is the fact.

*These sections are specific provisions which staff would include in those sections of Subpart B of the proposed rule where the Commission is not presently prepared to propose particular provisions. The Commission wishes to elicit comment on these provisions.

(c) An advertisement for a food which makes any representation permitted under § 437.6 (a) and (b) hereinabove shall not further represent that such food is nutritionally superior to any other food(s) on the basis of such permitted representation.

1. What, if any, is the proper definition of the term "natural food"? "Naturally grown food"? If any such definition exists for either term, is it generally accepted (among scientists or nutritionists; among consumers) or is there confusion, misunderstanding or disagreement about it?
2. What, if any, is the proper definition of the term "organic food"? "Organically grown food"? . . .
3. What empirical evidence, if any, is there of consumer understanding of the terms "natural," "naturally grown," "organic" and "organically grown"? If such evidence exists, does it show a consumer understanding consistent with any generally accepted definitions of those terms?
4. What evidence, if any, is available to demonstrate that so-called "natural" (or "naturally grown") or "organic" (or "organically grown") foods are in any way superior to other foods other than the extent, if any, to which they are superior by reason of the facts enumerated (and allowed to be claimed) in paragraphs 437.6(a) and (b) of the staff proposal on "Natural and Organic Food Claims"?
5. If the terms "natural" (or "naturally grown") and "organic" (or "organically grown") cannot be defined in a manner which will eliminate consumer misunderstanding, should their use in advertising be prohibited?
6. If the terms "natural" or "naturally grown" are to be prohibited in advertising, which, if any, of the following claims should be permitted where truthful:

(a) ". . . does not contain any artificial or synthetic preservatives."?

(b) ". . . does not contain any artificial or synthetic flavors."?

(c) "... does not contain any artificial or synthetic colors."?

(d) "... does not contain any (other) artificial or synthetic ingredients."?

What other claims, if any, should be allowed?

7. If the terms "organic" or "organically grown" are to be prohibited in advertising, which, if any, of the following claims should be permitted where truthful:

(a) "... has not been grown with (or subjected to) pesticides (or artificial fertilizers; or artificial conditioners)."? What other claims, if any, should be allowed?

8. If any specific factual claim referred to in issues 6 or 7 is to be permitted in advertising, should any further representation of nutritional superiority based on such claim be allowed?

. . . .

§ 437.7 CLAIMS FOR FOODS INTENDED TO BE COMBINED WITH OTHER FOODS.

(a) If, in order to prepare a food for consumption, it is necessary for a consumer to add to an advertised food and other food(s), characterizing ingredient(s) or component(s), as such ingredient(s) or component(s) is (are) defined in 21 CFR §102.1, that fact shall be clearly and conspicuously disclosed in any advertisement for such food.

(b) An advertisement for a food described in paragraph (a) hereinabove may represent that consumption of a serving of the combination provides a designated percentage of the U.S. RDA of each of the nutrients contained in a serving of such combination, subject to the provisions of subparagraph 437.2 (a) (2) of this Rule. However, a representation that the advertised food alone provides a designated percentage of the U.S. RDA of the nutrients which are contained in a serving of such combination shall not be made.

(c) If a serving of the food(s), ingredient(s) or component(s) with which an advertised food is (are) necessarily combined contributes more than 50 percent of the U.S. RDA of any nutrient named in the advertisement, it shall be clearly and conspicuously disclosed that most

of such nutrient is provided by such food(s), ingredient(s) or component(s).

(d) If an advertised food is frequently, but not necessarily, combined with any other food(s), ingredient(s) or component(s) for consumption, any representation regarding nutrition shall be based on the nutritional value of the advertised food alone.

1. Should a food which is sometimes but not necessarily combined with another food be allowed to disclose the nutrients present in the combination so long as the nutrients present in the advertised food alone are disclosed with equal prominence? If so, should such additional disclosure be limited to print advertising, or be allowed in other media as well?

§ 437.8 ENERGY AND CALORIE CLAIMS.

(a) An advertisement shall not represent that a food or nutrient contains, produces, provides, enhances, or is a source of "energy" or "food energy," or use any other word, demonstration or depiction of similar import, unless it clearly and conspicuously discloses, in immediate conjunction with the making of each such representation, that "energy" or "food energy" is supplied by calories, as well as the number of calories contained in a stated serving of the advertised food.

(b) An advertisement shall not represent that consumption of a food or nutrient, by itself, will produce or provide health, general vigor, sustained energy or alertness, or that the energy from calories, by itself, will produce or provide strength, endurance, intellectual performance, or the prevention or relief of fatigue.

(c) An advertisement shall not represent that consumption of a food in any way enhances or contributes to a person's vigor, energy, alertness, strength or endurance, unless it clearly and conspicuously discloses, in immediate conjunction with the making of each such representation:

(1) That such vigor, energy, alertness, strength or endurance is enhanced by and depends, in part, upon the calories in the food; and

(2) The number of calories contained in a stated serving of the advertised food.

(d) An advertisement shall not represent that consumption of any food or meal is useful

for, or contributes in any way to, or is useful in, regulating or maintaining caloric intake or body weight by the use of any demonstration or depiction, or any word or phrase such as "diet," "dietetic," "low calorie," "low in calories," "fewer calories," "calorie reduced," "contains artificial sweeteners," "artificially sweetened," or any other demonstration, depiction or term of similar import, unless:

(1) The advertised food complies with the provisions of 21 CFR §125.6; and

(2) The number of calories contained in a stated serving of the advertised food is clearly and conspicuously disclosed.

1. Is adequate and proper exercise a sufficiently useful way of altering daily caloric balance as to require the mention of this factor in advertisements for foods claiming to be useful in regulating or maintaining caloric intake or body weight?
2. Should advertisements for foods claiming to be useful in regulating or maintaining caloric intake or body weight be required to disclose, either alone or in addition to the disclosure presented in issue 1 above, that a reduction of total caloric intake should be combined with consumption of the advertised food?

(e) An advertisement for a food which makes any representation described in paragraph(d) hereinabove, and which contains any artificial sweetener, except one which serves an authorized technological purpose (as defined in 21 CFR §125.1(i)) shall comply with the provisions of subparagraphs (d) (1) and (2) hereinabove, and

(1) Shall clearly and conspicuously disclose the number of calories contained in a stated, equal-sized serving of the same food made with nutritive sweeteners; and

(2) If the artificially sweetened product contains a nutritive sweetener, the advertisement shall clearly and conspicuously make the following specific disclosure:

"This food contains sugars and should not be used by diabetics without the advice of a physician."

(f) An advertisement shall not represent that a food is "sugarless," "sugar free," "contains no sugar" or use any other term of similar

import, unless such food contains no sugars, including, but not limited to, sorbitol, mannitol, or other hexitol(s).

. . . .

*§ 437.9 FAT, FATTY ACID AND CHOLESTEROL CONTENT CLAIMS.

(a) An advertisement shall not contain any representation regarding the fat, fatty acid or cholesterol content of an advertised food, unless such food meets the criteria set forth in 21 CFR § 1.18 and carries a nutrient label in compliance with that regulation. If such food meets the criteria set forth in 21 CFR § 1.18 and carries a nutrient label in compliance with that regulation, an advertisement may contain only those representations which are permitted under 21 CFR § 1.18.

(b) An advertisement shall not contain any representation that consumption of an advertised food or a serving thereof will prevent, mitigate or cure, or in any way contribute to the prevention, mitigation or cure of, heart or artery disease or any attendant condition.

(c) An advertisement shall not represent that consumption of an advertised food or a serving thereof will not cause or help cause, or will not increase or help increase the likelihood or risk of, heart or artery disease or any attendant condition.

1. What claims, if any, concerning food and heart or artery disease or any attendant conditions or the fat, fatty acid or cholesterol content of food, which are forbidden by 21 CFR §1.18 to be made in food labeling, should be permitted in advertising? Why should such claims be permitted in advertising but not in labeling? Is any such claim, even if literally true, likely to carry with it any additional implication(s) which would be deceptive or unfair?
2. Is there any claim concerning food and heart or artery disease or any attendant condition which is not forbidden by 21 CFR § 1.18 and which should be allowed in food advertising?

*These sections are specific provisions which staff would include in those sections of Subpart B of the proposed rule where the Commission is not presently prepared to propose particular provisions. The Commission wishes to elicit comment on these provisions.

*§ 437.10 HEALTH AND RELATED CLAIMS.

(a) An advertisement shall not contain a representation that:

(1) A food, because of the presence or absence of certain vitamins and/or minerals, is adequate or effective in the prevention, cure, mitigation, or treatment of any disease or symptom.

(2) A balanced diet of ordinary foods cannot supply adequate amounts of nutrients.

(3) The lack of optimum nutritive quality of a food, by reason of the soil on which that food is grown, is or may be responsible for an inadequacy or deficiency in the quality of the daily diet.

(4) The storage, transportation, processing or cooking of a food is or may be responsible for an inadequacy or deficiency in the quality of the daily diet.

(5) A food possesses any dietary property when such property is of no significant value or need in human nutrition. Ingredients or products such as rutin, or other bioflavonoids, para-aminobenzoic acid, inositol and similar substances which have not been shown to be essential in human nutrition shall not be represented in any way which states or implies nutritional benefit. Ingredients or products of this type may be advertised as individual products or mixtures thereof; *Provided, however*, that the possibility of nutritional dietary or therapeutic value is not stated or implied. Examples of false or misleading statements or implications are:

(i) Statements to the effect that their need or usefulness in human nutrition has not been established.

*These sections are specific provisions which staff would include in those sections of Subpart B of the proposed rule where the Commission is not presently prepared to propose particular provisions. The Commission wishes to elicit comment on these provisions.

(ii) Statements which otherwise disclaim nutritional, dietary or therapeutic value.

(6) A natural vitamin in a food is superior to an added or synthetic vitamin, or that there is a difference between vitamins, naturally present and those that have been added.

(b) A food shall not be represented in advertising as a "health food" or as containing "health foods," or otherwise be depicted, described or designated by any term or demonstration of similar import.

1. What claims, if any, concerning the nutritive or medical benefits allegedly resulting from the consumption of foods which are forbidden to be made in food labeling by 21 CFR § 1.17(i) should be permitted in advertising? Why should such claims be permitted in advertising but not in labeling? Is any such claim, even if literally true, likely to carry with it any additional implication(s) which would be deceptive or unfair?
2. What, if any, is the proper definition of the term "health food"? If such a definition exists, is it generally accepted (among scientists or nutritionists; among consumers) or is there confusion, misunderstanding or disagreement about it?
3. What empirical evidence, if any, is there of consumer understanding of the term "health food"? If such evidence exists, does it show a consumer understanding consistent with any generally accepted definitions of those terms?
4. If the term "health food" cannot be defined in a manner which will eliminate consumer misunderstanding, should its use in advertising be prohibited?

Recent Awards in Nutrition

At the annual convention of the American Medical Association, three of four major awards were made to physicians who have made major contributions to the field of clinical nutrition.

Stanley Dudrick, M.D., Director of Surgery at the University of Texas Medical School, Houston, received the Brookdale Award in Medicine given to physicians under the age of fifty. Dr. Dudrick was cited for his role in developing intravenous hyperalimentation.

Ananda S. Prasad, M.B., Ph.D., Chief of Hematology at Wayne State University, Detroit, received the Joseph B. Goldberger Award for Clinical Nutrition. Dr. Prasad was cited for research on dysproteinemias, hematological disorders, and trace mineral metabolism. Dr. Prasad is a pioneer in the description of human zinc deficiency and is a leader in the field of trace element metabolism. He is editor of the two-volume Nutrition Foundation monograph, currently in press, entitled "Trace Elements and Human Disease."

Rudolph H. Kampmeier, M.D., Professor of Medicine Emeritus at Vanderbilt University, received the Dr. Rodman E. Sheen and Thomas E. Sheen Award, which is presented to a U.S. physician for scientific accomplishment. This is the top award of the American Medical Association. Dr. Kampmeier was cited for his career as a dedicated physician, teacher, communicator and leader. Dr. Kampmeier's balanced career interests have included a continuing involvement in the field of clinical nutrition. He served the U.S. Armed Forces in a consultant capacity during and following the Second World War and as a senior member of a number of the nutrition survey teams in Latin America and the Middle East, under the auspices of the Interdepartmental Committee on Nutrition for National Defense (ICNND). His thoughtful argument for improvement of the teaching of nutrition in medical schools was set forth in a signed editorial in the Southern Medical Journal.

New FAO Periodical

"Food and Nutrition," a publication covering world developments in food policy and nutrition has been inaugurated by the Food and Agriculture Organization (FAO). The new review, to be published quarterly, will replace the FAO's "Nutrition Newsletter."

"Food and Nutrition" will be prepared by the Food Policy and Nutrition Division of FAO. Most of FAO's activities are directed toward the production of and trade in foods and other commodities. The Food Policy and Nutrition Division's role is to integrate nutritional objectives into these activities.

In announcing the inauguration of the periodical, which is dedicated to Lord

Boyd Orr, first Director General of the FAO, Director General A. H. Boerma said, "the activities of the Food Policy and Nutrition Division will acquire significantly increased relevance in the light of the World Food Conference and the marked attention it paid to the continuing blight of widespread hunger and malnutrition in the world." The entire first issue is devoted to the World Food Conference.

The periodical will be published in English, French and Spanish. Subscriptions and inquiries are to be addressed to: Distribution and Sales Section, FAO, Via delle Terme di Caracalla, 00100, Rome, Italy. Price per copy, Lit. 1650 (\$2.64); per year, Lit. 5200 (\$8.32).

Recent Books

Immobilized Enzymes, Antigens, Antibodies, and Peptides. Edited by H. Weetall. Published by Marcel Dekker, Inc., 270 Madison Avenue, New York, New York 10016. Pp. 661. Price \$38.50.

The Mayo Clinic Renal Diet Cookbook, by J. C. Margie, C. F. Anderson, R. A. Nelson and J. C. Hunt. Published by Western Publishing Company, Inc., 850 Third Avenue, New York, New York 10022. Pp. 307. Price \$10.00. This book provides the physician and his patient with basic diet plans of great flexibility. Many recipes are provided.

The Promotion of Bottle Feeding by Multi-national Corporations: How Advertising and the Health Professions Have Contributed, by T. Greiner. M. C. Latham, Editor. Cornell International Nutrition Monograph Series, No. 2. Published by Cornell University. Copies may be obtained from: Dr. Michael C. Latham, Division of Nutritional Sciences, Savage Hall, Cornell University, Ithaca, New York 14853. Pp. 82. Price. \$2.00.

The Effect of Soils and Fertilizers on Human and Animal Nutrition, by W. H. Allaway. Agriculture Information Bulletin No. 378. Published by Superintendent of Documents, U.S. Government Printing Office, Washington, D.C. 20402. Pp. 52. Price \$.80.

Applied Nutrition, by R. Rajalakshmi. Second edition. Published by Mohan Primlani, Oxford & IBH Publishing Co., 66 Janpath, New Delhi 110001, India. Pp. 548. Price \$3.63.

Body Dimensions and Proportions, White and Negro Children 6-11 Years, United States. Vital and Health Statistics, Series 11, No. 143, DHEW Publication No. (HRA) 75-1625. Published by Health Resources Administration, DHEW, Rockville, Maryland 20852. Available from Superintendent of Documents, U.S. Government Printing Office, Washington, D.C. 20402. Pp. 66. Price \$1.30.

Meeting Announcements

The American Diabetes Association will be sponsoring two activities in October, 1975. A meeting on Perspectives in Current Diabetes Research will be held October 16-17, 1975. The First Combined Health Care Professionals Course in Diabetes will be held October 17-19, 1975. For further information concerning either event contact:

Ernest M. Frost, Ed.D.
Executive Vice-President
1 West 48th Street
New York, New York 10020

Special Notice

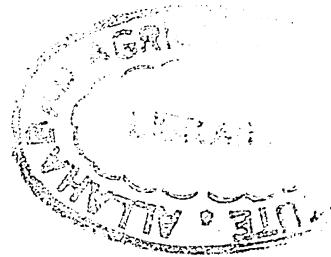
All subscription inquiries, renewal requests and change of address notices for NUTRITION REVIEWS should be sent to:

The Nutrition Foundation
Office of Education
888 Seventeenth Street, N.W.
Washington, D. C. 20006

12 NOV 1975

Silicon

by Edith M. Carlisle, Ph.D.



The discovery of the "newer" essential trace elements in recent years has encouraged reconsideration of elements that were previously thought to be environmental contaminants. Silicon is one of these elements and it occupies a unique position. Next to oxygen, silicon is the most prevalent element on earth, and crystalline silica in the form of quartz is the most abundant mineral in the earth's crust. To label it a trace element may require some adjustment in thinking.

Although interest in the silica content of animal tissues and the effects of siliceous substances upon animals was evidenced over half a century ago, emphasis has been placed on the more deleterious aspects of silicon metabolism. These include its effect upon forage digestibility, urolithiasis and especially pneumoconioses (silicosis) caused by dust inhalation. Several important reviews on this work are available.¹⁻³

The element occurs in nature as the oxide silica (SiO_2) or the corresponding silicic acids formed by the hydration of the oxide. Orthosilicic acid ($\text{Si}(\text{OH})_4$) is the simplest acid and the main form soluble in water in amounts up to about 120 ppm. Supersaturation causes it to dehydrate and polymerize into complex and less soluble forms.

Considering its slight solubility in water and its presence in most plants, it is not

surprising that at least minute amounts of silicon may be found in most animal tissues and fluids. Nevertheless, the wide range of silicon content reported in animal tissues by some of the early French and German investigators probably reflects interference by phosphorus in the analytical method used (earlier work reviewed by King and Belt¹). Microdetermination of silicon in biological tissues has been reported to be one of the most difficult problems in analytical chemistry. A satisfactory general colorimetric method has been developed for most biological tissues, of which there are several appropriate modifications.

The highest levels of silicon are found in the epidermis and its appendages, and in connective tissues in general. The eggs of birds, milk and the fetuses of mammals have small but appreciable quantities. The blood of man and other mammalian species averages about 5 ppm, a level that is not significantly increased by the inhalation of silica dust. Dietary silicon supplements have been reported⁴ to have little effect on the silicon concentration of cow's milk. Moderate increases have been obtained in rat's blood, however, after feeding silicon as sodium metasilicate and much higher levels have been reached after feeding organic silicates.⁵ The blood appears capable of maintaining a considerably higher concentration of organic than of inorganic silicate. The consistently low concentration of silica in most organs does not appear to vary appreciably during life. Parenchymal tissues such as liver, heart and muscle, for example, range from 2 to 10 ppm. The lungs are an exception. Varying amounts of silica entering the respiratory tract normally cross the barrier of the lung as silicic acid which is eventually eliminated. Never-

Dr. Carlisle is Associate Researcher in Nutritional and Environmental Sciences at the University of California School of Public Health, Los Angeles, California 90024.

A limited number of reprints of this article may be obtained from the author. THERE ARE NO REPRINTS OF UNSIGNED REVIEWS.

theless, the lungs ordinarily accumulate large amounts of silicon from long continued inhalation of finely particulate silica.

Silicic acid in foods and beverages is readily absorbed across the intestinal wall and is rapidly excreted in the urine. Recent studies⁶ on silicon absorption using intestinal ligature techniques showed that the level of silicon in blood and intestinal tissues of male and female rats is affected by age, sex, castration, adrenalectomy and thyroidectomy.

Essential Element

A series of experiments contributed to the establishing of silicon as an essential element beginning with *in vitro* studies on young bone, suggesting a physiological role for silicon in bone calcification processes. This was followed by *in vivo* studies showing an effect of silicon on the rate of bone mineralization. Of paramount importance, however, was establishing a deficiency state incompatible with normal growth, and the contrasting normal growth on a diet containing a silicon supplement, reported in 1972.^{7,8} Feeding a low silicon diet based on an optimal mixture of L-amino acids for the chick and using special trace element techniques, it was possible to show that silicon is required for normal growth and development. Increases of nearly 50 percent in growth rates in chicks were observed upon the feeding of silicon supplied as sodium metasilicate. The chicks on the deficient diet appear stunted. On subsequent examination all organs appeared relatively atrophied. Macropathologic examination showed that the skin and mucous membranes were somewhat anemic. The deficient chick had no wattles and the comb was severely attenuated. Significantly retarded skeletal development was also evidenced by reduced circumference, thinner cortex and less flexible leg bones. The skulls were also smaller and abnormally shaped with the cranial bones appearing flatter. This effect of silicon on skeletal development supported earlier

findings that silicon is involved in an early stage of bone formation.⁹

Silicon deficiency in rats also results in depressed growth and skull deformities.¹⁰ Chemically defined diets based on amino acids in place of protein were also used. The addition of 50 mg of silicon per 100 g of diet produced a 25 to 34 percent increase in growth rates. The skulls were shorter and the bone structure surrounding the eye appeared distorted. Pigmentation of the incisors was also affected. Silicon was only partly effective in preventing the impairment of pigment deposition. Significant effects on the incisor pigmentation were also produced by physiological levels of tin, vanadium or fluorine.

Silicon, Calcium and Bone Calcification

Earlier studies suggested a physiological role for silicon on bone calcification. *In vitro* studies^{11,12} showed the unique localization of silicon in active calcification sites in young bone. Furthermore, in the earliest stages of calcification in these sites, when the calcium content of osteoid tissue is very low, a direct relationship exists between silicon and calcium. Neither the initiating nor limiting factor in the mineralization of bone in the living animal is known. It has been thought that crystallization in matrices must occur on sites which form specific nucleation centers. Several recent investigators regarded calcium binding as a most important and first event in calcification. It was suggested that silicon may be associated with calcium in this process.

Subsequent *in vivo* experiments with weanling rats^{1,2} also showed a relationship between silicon and calcium in bone formation. These experiments demonstrated that dietary silicon increases the rate of mineralization; this effect was particularly apparent under conditions of low calcium intake. A somewhat similar mechanism of action (in bone formation) has been demonstrated for vitamin D.¹³ A relationship has also been established between silicon, calcium, magnesium and fluorine in bone formation in the chick.¹⁴

Mucopolysaccharides

A necessity for silicon has been established in articular cartilage and connective tissue formation. Skeletal and other abnormalities involving mucopolysaccharides in formation of the cartilage matrix and connective tissue were found to be associated with silicon deficiency in the chick.^{15,16} Tibial-metatarsal and tibial-femoral joints are smaller in the silicon-deficient chicks. The ends of the bones had less articular cartilage and were not as well formed. Analysis revealed that the bones of the deficient group had 35 percent less water and a significantly decreased proportion of mucopolysaccharides in the articular cartilage. Both the amount of cartilage and mucopolysaccharide content was considerably less in the silicon-deficient chicks. The total amount of collagen formed was also reduced. This same relationship established between silicon and mucopolysaccharides in cartilage formation was confirmed in another type of connective tissue, the "target" connective tissue, the cock's comb.

Further support for a role of silicon in mucopolysaccharide metabolism is the finding that silicon is an integral component of animal mucopolysaccharides and their protein complexes. The site of action of silicon in connective tissue metabolism is in the mucopolysaccharide-protein complexes of the ground substance. In higher animals the mucopolysaccharides, hyaluronic acid, chondroitin sulfates and keratan sulfate are found to be linked covalently to proteins as components of the extracellular, amorphous ground substance that surrounds the collagen, elastic fibers and the cells. By extraction and purification of several connective tissues, silicon has been shown to be chemically combined in the mucopolysaccharide fraction. The silicon content of the mucopolysaccharide protein complex extracted from bovine nasal septum, for example, is 87 ppm compared to 13 ppm in the original dried cartilaginous tissue. Analyses of individual purified mucopolysaccharides¹⁷ show relatively high amounts of silicon in

chondroitin sulfate A, dermatan sulfate, heparan sulfate and hyaluronic acid from sources such as the umbilical cord, and lesser amounts in chondroitin sulfate C, heparan and keratan-2 sulfate from cartilage. On the other hand hyaluronic acid from vitreous humor and keratan-1 from cornea are very low. Silicon appears to be covalently bound to the polysaccharide matrix in these mucopolysaccharides probably in an ester linkage, C-O-Si.^{16,17} The possibility of a polysaccharide-Si-ester linkage existing in biological material has also been reported by Heinen.¹⁸

The findings have important implications because apart from bone, cartilage and connective tissue formation, silicon must also participate in other processes in which mucopolysaccharides are involved. This includes growth and maintenance of connective tissues, as in embryonic development and wound healing. Degenerative conditions (such as atherosclerosis and osteoarthritis) and the overall aging process are also associated with significant changes in mucopolysaccharides.

Aging

It is not surprising then to find a relationship between silicon and aging in certain tissues. The silicon content of the aorta, skin and thymus is found to decline significantly with age. This contrasts with other tissues such as the heart, kidney, muscle and tendon which show little or no change. This relationship occurs in several species, including the rabbit, rat, chicken and pig.¹⁶

Similarly, in human skin, the silicon content of the dermis has been stated to diminish with age.^{19,20} In contrast with an earlier finding,²¹ French investigators²² reported that the silicon content of the normal human aorta decreases considerably with age and, furthermore, that the level of silicon in the arterial wall decreases with the development of atherosclerosis. Of possible significance here is the report of another French worker⁶ on changes in absorption and resulting levels of silicon in the blood and intestinal tissues of rats in

relation to age, sex and various endocrine glands. It is suggested that the decline of hormonal activity in senescence may well account for the modifications in silicon observed in aged animals.

Collagen Component

Silicon is also shown to be a component of collagen to which some mucopolysaccharides are attached. Because reduced amounts of collagen were found in silicon-deficient chicks, in vitro studies were undertaken to further investigate this silicon/collagen relationship.^{2,3} Since the maturation and age of collagen is related to its solubility, undenatured collagen of young skin was completely fractionated into various solubility classes. Silicon was shown to be a component of collagen of all four fractions, suggesting that silicon may play a fundamental role in cross linking mechanisms of collagen formation. Analyses of a variety of collagens from different sources also showed silicon to be a bound component.^{2,4}

In connective tissues the unique properties of silicon atoms which lend themselves to macromolecular structure are evident. Silicon serves as an important crosslinking agent by forming links or bridges within and between individual polysaccharide chains and linking polysaccharide chains to proteins. In this way, silicon aids in the development of the architecture of the fibrous elements of connective tissues and contributes to its structural integrity by providing strength and resilience.

In very recent studies, the cellular localization of silicon has been demonstrated in the active bone cell, the osteoblast.^{2,5,26} Bone formation depends on the activity of osteoblasts which synthesize the organic matrix and presumably modify the calcification process. Evidence is accumulating to support the view that the osteoblasts, in addition to controlling matrix synthesis, also regulate the exchange of ions between the bulk extracellular fluids and those of mineralizing bone.^{2,7} X-ray microanalysis of active growth areas in young bone and isolated osteoblasts and further studies in-

cluding ultracentrifugation demonstrate that silicon is concentrated in the cytoplasm of the osteoblast in the mitochondria. A quantitative relationship is established between silicon and calcium in the mitochondria where calcium is also concentrated. The accumulation of silicon and calcium in the cell and mitochondria occurs before any evidence of extracellular ossification. These findings add further support to the proposal that silicon plays a role in the initiation of the calcification process.

Although the need for silicon is demonstrated for laboratory animals, there is no known corresponding need in man. It is probable, however, that silicon may have considerable implications in human nutrition, as past experience with other trace elements has demonstrated. □

1. E. J. King and T. A. Belt, *Physiol. Rev.* 18: 329-365, 1938
2. L. A. P. Jones and K. A. Handreck, *Adv. Agron.* 19: 107-149, 1967
3. *Trace Elements in Human and Animal Nutrition* by E. J. Underwood. Third edition. Academic Press, New York, 1971
4. J. C. Archibald and H. Fenner, *J. Dairy Sci.* 40: 703-706, 1957
5. E. M. Carlisle, unpublished observations
6. Y. Charnot and G. Peres, *Ann. Endocrinol. (Paris)* 32: 397-402, 1971
7. E. M. Carlisle, *Fed. Proc.* 31: 700, 1972
8. E. M. Carlisle, *Science* 178: 619-621, 1972
9. E. M. Carlisle, *Science* 167: 279-280, 1972
10. K. Schwarz and D. B. Milne, *Nature (London)* 239: 333-334, 1972
11. E. M. Carlisle, *Fed. Proc.* 28: 374, 1969
12. E. M. Carlisle, *Fed. Proc.* 29: 565, 1970
13. S. A. Muller, A. S. Posner and H. E. Firschein, *Proc. Soc. Exp. Biol. Med.* 121: 844-846, 1966
14. E. M. Carlisle, *Fed. Proc.* 30: 462, 1971
15. E. M. Carlisle, *Fed. Proc.* 32: 930, 1973
16. E. M. Carlisle, *Fed. Proc.* 33: 1758-1766, 1974
17. K. Schwarz, *Proc. Nat. Acad. Sci. USA* 70: 1608-1612, 1973
18. W. Heinen, *Arch. Biochem.* 110: 137-149, 1965
19. H. Brown, *J. Biol. Chem.* 75: 789-794, 1927

20. R. C. MacCardle, M. F. Engman, Jr. and M. F. Engman, Sr., *Arch. Dermat. Symp.* 47: 335-372, 1943
21. S. A. Kvorning, *J. Gerontol.* 5: 23-25, 1950
22. J. Loeper, J. Loeper and A. Lemaire, *Presse Med.* 74: 865-868, 1966
23. E. M. Carlisle, *Fed. Proc.* 33: 704, 1974
24. K. Schwarz and S. C. Chen, *Fed. Proc.* 33: 704, 1974
25. E. M. Carlisle, *Fed. Proc.* 34: 927, 1975
26. E. M. Carlisle in *Proceedings of the Tenth International Congress of Nutrition*, Vol. VIII (in press)
27. H. Rasmussen, J. Feinblatt, N. Nagata and M. Pechet, *Fed. Proc.* 29: 1190-1197, 1970

Nutrition Reviews

authoritative articles by distinguished international scientists • competent, critical, and knowledgeable reviews • interpretative summaries of important original clinical and experimental research • selected excerpts from classic publications marking milestones in nutrition research • informative special reports on government and organization actions • contributing editors review journals of widespread interest covering biochemistry, food science, public health, toxicology, dentistry, pediatrics, internal medicine, gastroenterology and other clinical specialties •

Send subscription and renewal requests to:

THE NUTRITION FOUNDATION
888 Seventeenth Street, N.W.
Washington, D. C. 20006

\$12.00 per year

\$6.00 for students

THE SOMATOMEDINS AND GROWTH

Recent studies showed that there are a number of somatomedins with different chemical and physiological properties. One of the somatomedins is reduced in renal failure and returns to normal after kidney transplantation.

Key Words: somatomedin, sulfation factor, growth hormone, catch-up growth, renal failure, dental development.

Despite the appreciation that the pituitary is necessary for normal growth since the turn of the century, pituitary growth hormone (GH) was first isolated only 30 years ago.¹ Subsequent work demonstrated that GH is unusual among polypeptide hormones in showing marked species specificity. The effect of GH on the growth of skeletal tissue is also not by GH itself but by some other biologically active factor which has been descriptively named sulfation factor (SF)² because of its stimulatory action on the incorporation of radioactive sulfate by the costal cartilage of hypophysectomized rats. With increasing sophistication of analytic and chemical techniques it has become apparent that there is not one but several GH-dependent serum factors. Recently the term somatomedin (SM) has been proposed as a generic term for any GH-dependent substance that mediates the action of GH.³ To date at least three somatomedins have been described with different chemical and biologic properties. Somatomedin A stimulates sulfate uptake by chick cartilage. Somatomedin B stimulates the growth of human glia cells in vitro. Somatomedin C is a more basic peptide than A or B and stimulates rat cartilage sulfate incorporation.

Hall and Filipsson⁴ recently reported on the correlation between somatomedin A in serum and the somatic growth of both normal and abnormal children. The serum somatomedin concentration is determined

by bioassay. The technique involves the measurement of the incorporation of radioactive sulfate into embryonic chick cartilage and the comparison to an arbitrary reference standard from one healthy adult male. Sixty-six children were studied in all. Seventeen were selected at random from a larger group of healthy school children. They were examined annually from seven to 16 years for their anthropometric development. The remaining children were divided into three groups on the basis of their height at the age of eight years. Twenty-nine were short, being more than two standard deviations below the appropriate mean. They included 12 pituitary dwarfs and a variety of other diagnoses. Nine children were tall by analogous criteria but were otherwise healthy. The remainder were of normal stature but could not be defined as healthy because they had been admitted to the hospital for investigation of tall stature, delayed puberty or hypogonadism.

Although the children were measured repeatedly for a number of years only one serum somatomedin on each child was reported. The age at which the blood sample was taken was not stated although the authors report a significant negative correlation of somatomedin with age. They appreciate the invalidity of mixing different clinical groups, however, because they point out that the hypopituitary children were both the oldest and those with the lowest serum somatomedin. When they were omitted from analysis, no correlation of somatomedin with age was observed.

An interesting concept of dental maturity was used in other analyses. The eruption of the permanent teeth was measured repeatedly. The chronological age (A) at which a reference point on the eruption curve was reached was used for correlation with other variables. At age A all children were of equivalent dental maturity. Therefore the function $1/A$ is an estimate of the velocity of dental development. Serum somatomedin levels correlated very well ($P < 0.001$) with $1/A$ whether or not GH-deficient children were included in the analysis.

The authors reported that mean somatomedin levels in the children who were defined as short, normal or tall at the chronological age of eight years differed significantly. When height at eight or nine chronological years of age was plotted against somatomedin there was a positive correlation. Somatomedin also correlated positively with the growth velocity in the chronological year before the reference point of dental development was achieved. The biological significance of these observations is hard to interpret because of the mixed clinical nature of the sample studied and the fact that the serum in which the somatomedin was measured was taken at an undefined and often very different age than the measurements with which it was being correlated. One appreciates the problems facing an investigator wishing to study something so difficult to measure as somatomedin. Until more basic facts are established, however, such as the variation of somatomedin with age, cross sectionally and longitudinally, in a sample of normal children, data such as those presented by Hall and Filipsson remain difficult to interpret.

The association between dental and height development is interesting. It may be further used with profit as an index of growth in subjects with stunted growth perhaps for orthopedic reasons. The possibility also exists that dental development may be a more precise marker of growth than bone age, at least in the second half of childhood. If the association between dental

velocity and serum somatomedin is substantiated it may indicate that somatomedin levels, unlike those of GH, are relatively constant within an individual and that a random value gives a measure of the individual's growth rate over a number of years.

Another study by Saenger and his colleagues⁵ investigated the effects of treatment of endstage renal disease on growth and somatomedin levels in a group of nine boys between the ages of seven to 16 years. This was a complicated type of patient to study as growth failure in renal disease is known to be partly nutritional⁶ and partly related to immunosuppressive therapy after transplantation. Nevertheless children receiving renal transplants are of special interest as the kidney is one of the postulated sites of somatomedin production. At the time of study the boys had survived between nine months and five years after transplantation. All were receiving prednisolone and azothioprine maintenance therapy.

The renal response to transplantation was variable. Four boys had good kidney function during the post-transplant year in which growth was measured with creatinine clearance values between 40 and 90 ml per minute per 1.73 m^2 . Another four were characterized by low creatinine clearances. The last boy had a biphasic response with good renal function for two years followed by graft rejection. At the time of transplant all the children were growth retarded, being on or below the third percentile. Post-transplant growth was measured as a function of chronological age and velocity as a function of bone age at the time of study. Growth rate increased after the operation in four and remained unchanged in four others. It followed a biphasic pattern in the boy who had a biphasic renal response. Growth and renal improvement did not match exactly; one child had poor renal function in the accelerated growth group and vice versa.

Serum somatomedin activity was measured by the in vitro uptake of radioactive sulfate by pieces of costal cartilage from

hypophysectomized rats using a pool of serum from ten healthy adult males as a reference standard. The authors appreciated that high inorganic sulfate levels in uremic sera would influence their assay results and made corrections for this variable. They did not consider the possible effects of other metabolites or drugs such as the steroids which might be expected to affect incorporation of sulfate by cartilage. Despite these problems the results of serum somatomedin assay were remarkably uniform. They were reduced in all boys pre-transplant (mean \pm ISD, 0.39 ± 0.10 units per milliliter) and rose after transplantation 65 percent or more to values within the normal range (0.84 ± 0.15). The post-transplant somatomedin level did not correlate significantly with creatinine clearance suggesting that renal function does not have a controlling effect on serum somatomedin. Somatomedin levels did correlate positively with growth rate post-transplantation. The complexity of the interrelationships is illustrated by the fact that growth velocity also correlated with creatinine clearance, however, it correlated negatively with the prednisolone dosage. The nine boys had normal growth hormone responses to arginine plus insulin stimulation but neither basal nor maximum plasma growth hormone levels were related to growth rate.

Besides illustrating the complexity of growth control in renal disease, this study demonstrated for the first time the depression of serum somatomedin in renal failure and its normalization following transplantation. The authors concluded with justification that the growth failure of endstage renal disease may be due in part to low serum somatomedin activity. The restoration of somatomedin after transplant is a necessary but not a controlling factor for the resumption of growth.

A methodological study by Yalow and her co-workers⁷ demonstrates again the complexity of this group of molecules in both their biologic characteristics and their measurement: In describing a radioimmunoassay for somatomedin B the

authors also clearly indicate the direction in which further work in this field is needed. Somatomedin B is a somatomedin which has similar chemical properties to somatomedin A but stimulates DNA synthesis in human glia like cells. Small amounts were obtained from human plasma through a human growth hormone extraction laboratory. They were used to raise antisera, as standards and to prepare radioiodine-labeled somatomedin. A conventional radioimmunoassay was then developed with charcoal separation. This was capable of detecting approximately 25 pg immunoreactive somatomedin B per milliliter. Because of the normal plasma concentration in man, the assay is capable of detecting somatomedin in normal human plasma at dilutions of 1:20,000 or greater. No immunoreactive material was found in guinea pig, mouse, rat, cow, dog, sheep, pig or rabbit plasmas diluted 1:20. Somatomedin B was detected, however, in monkey plasma diluted 1:5000. In 12 normal human male subjects the fasting plasma somatomedin B level ranged from 4 to 20 μ g per milliliter. Radioactive somatomedin B did not bind to antisera raised against insulin or growth hormone. No immunoreactive somatomedin B was detected in samples of somatomedin A, C or nonsuppressible insulin-like activity (NSILA). Acromegalic subjects had levels of somatomedin B approximately three times greater than those of hypopituitary patients.

Gel filtration of unextracted human plasma revealed that somatomedin B eluted in the region of gamma globulin. On paper electrophoresis both purified somatomedin B and somatomedin B added to guinea pig plasma migrated in the intra- α -globulin region. Starch gel electrophoresis of human plasma showed, however, most of the endogenous somatomedin B remaining at the origin indicating its binding to a human serum protein. The sedimentation characteristics of somatomedin B in plasma on ultracentrifugation also indicate that it is bound to a gamma globulin. In contrast, purified somatomedin B added to guinea

pig plasma sedimented more slowly than labeled albumin and at a rate similar to that of insulin.

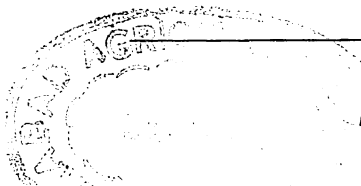
Turnover studies were performed by injecting unextracted human plasma somatomedin B from gel filtration intravenously into a dog. Plasma removed from the animal at intervals after injection had somatomedin B elution characteristics similar to those of the original human plasma indicating no appreciable binding by canine serum proteins. Somatomedin B did not have a simple exponential clearance from the dog's circulation but the 30 to 90 minute postinjection period approximated an exponential decay with a half-life of 50 to 60 minutes.

These findings allow a number of interesting deductions to be made. A central question concerning somatomedins is whether they are subunits of growth hormone or molecules made in other cells such as hepatocytes under the influence of growth hormone. The failure of somatomedin B to react with growth hormone antisera does not mean that it is necessarily a separate molecule as the antigenic sites of growth hormone might be destroyed or distorted during transformation into somatomedin. The persistence of somatomedin B in the serum of hypopituitary subjects in whom growth hormone is undetectable, however, is in favor of it being synthesized from a different source. Further evidence for this comes from the relative plasma concentrations of somatomedin B and growth hormone. The thousand-fold greater concentration of somatomedin would be compatible with its origin in growth hormone only if the turnover time were many times longer than that indicated from the canine study.

The paper of Yalow and her colleagues clearly shows how research on the somatomedins is inhibited until an assay system with the speed, precision and sensitivity of a radioimmunoassay is developed. With such a tool not only can essential biochemical

information be gathered relating to the size, association and kinetics of a somatomedin but the tool is available to measure fundamentals such as the change in plasma somatomedin as a function of age and sex in normal individuals. Studies based on bioassay, such as the first two reviewed here, are subject to a variety of problems including the difficulty of comparing the results of one laboratory with those of another. They do have a limited use, however, in the collection of qualitative and semiquantitative information such as the changes in the same person as a result of renal transplantation. □

1. C. H. Li and H. M. Evans: The Isolation of Pituitary Growth Hormone. *Science* 99: 183-184, 1944
2. W. D. Salmon, Jr. and W. H. Daughaday: A Hormonally Controlled Serum Factor which Stimulates Sulfate Incorporation by Cartilage in Vitro. *J. Lab. Clin. Med.* 49: 825-836, 1957
3. W. H. Daughaday, K. Hall, M. S. Raben, W. D. Salmon, Jr., J. L. Van den Brande and J. J. Van Wyk: Somatomedin: Proposed Designation for Sulphation Factor. *Nature* (London) 235: 107, 1972
4. K. Hall and R. Filipsson: Correlation between Somatomedin A in Serum and Body Height Development in Healthy Children and Children with Certain Growth Disturbances. *Acta Endocrinol.* 78: 239-250, 1975
5. P. Saenger, E. Wiedemann, E. Schwartz, S. Korth-Schutz, J. E. Lewy, R. R. Riggio, A. L. Rubin, K. H. Stenzel and M. I. New: Somatomedin and Growth after Renal Transplantation. *Pediat. Res.* 8: 163-169, 1974
6. J. M. Simmons, C. J. Wilson, D. E. Potter and M. Holliday: Relation of Caloric Deficiency to Growth Failure in Children on Hemodialysis and the Growth Response to Calorie Supplementation. *New Engl. J. Med.* 285: 653-656, 1971
7. R. S. Yalow, K. Hall and R. Luft: Radioimmunoassay of Somatomedin B. Application to Clinical and Physiologic Studies. *J. Clin. Invest.* 55: 127-137, 1975



12 NOV 1975

ZINC DEFICIENCY IN SICKLE CELL DISEASE

Clinical and biochemical symptoms of zinc deficiency are found in sickle cell disease. The symptoms respond to large doses of oral zinc suggesting that zinc supplementation may be beneficial in this disease.

Key Words: sickle cell anemia, zinc deficiency, zinc therapy

The severity of the anemia of sickle cell disease depends on such factors as the kind and amounts of abnormal hemoglobin in each red blood cell, the total size of the red cell population and the number of cells which are capable of sickling. Oelshlegel et al.¹ showed that zinc can increase the oxygen affinity of both normal and sickle red blood cells, possibly through mediation of a hemoglobin binding mechanism. Thus, a deficiency of zinc may intensify the effects of the anemia of sickle cell disease.

It has been suggested that some patients with sickle cell anemia (SCA) are zinc deficient. They have low red blood cell zinc concentrations,² possibly as a result of continuous hemolysis since zinc is present in high concentrations in erythrocytes. Prasad et al.³ noted that patients with SCA generally exhibit many clinical features similar to those observed in zinc deficiency. Zinc nutriture was examined in 18 men and 18 women, between the ages of 15 to 69 years, in whom diagnosis of SCA was established by history, physical examination and hematological studies. Hypogonadism and growth retardation were commonly seen in the SCA patients. Thirteen of 18 men with SCA manifested signs of hypogonadism, characterized by decreased or absent facial, axillary, chest and pubic hair and delayed onset of puberty. Only four females showed signs of gonadal dysfunction as indicated by retarded onset of menarche and menstrual abnormalities. Growth retardation and weight deficit as compared with data from the Ten State Nutrition Survey were observed in all the men and in 14 women.

Erythrocyte zinc concentrations of SCA patients were significantly lower, 35.2 μg , than those of controls, 41.7 μg per gram hemoglobin. Four SCA patients over 30 years of age had normal values. Levels of carbonic anhydrase, a zinc metalloenzyme whose synthesis is dependent upon zinc availability, correlated significantly with erythrocyte zinc levels.

Plasma zinc concentrations were also significantly lower in SCA patients as compared with controls (102 μg per 100 ml and 112 μg per 100 ml, respectively) as was the zinc concentration in the hair. Plasma ribonuclease activity was significantly higher in SCA patients as compared with controls, as would be expected in zinc deficiency.

In contrast, these patients who should have zinc-depleted tissues, had much higher urinary zinc excretion than controls; 739 μg per gram creatinine and 495 μg per gram creatinine, respectively. The authors suggest that increased amounts of zinc were in the filtrate arising from continued hemolysis. However, plasma zinc was low in these subjects, and thus, it seems unlikely that increased filtration of zinc could cause such an increased urinary excretion. Impaired tubular reabsorption or increased tubular secretion of zinc seem to be more likely explanations. Anemia, per se, was not responsible for the apparent zinc deficiency since patients with iron and folate deficiencies had significantly higher plasma and erythrocyte zinc concentrations than the controls. No explanation for these higher values was given by the authors.

A limited test of zinc therapy was conducted involving daily oral administration of zinc sulfate (660 mg, more than 20

times the RDA for zinc) to seven men and two women with SCA. Two 17-year-old males gained in height during zinc therapy; one gained 5 cm in 49 weeks and the other 7 cm in 42 weeks. Eight of these nine patients also gained in weight with only one patient, a female, losing 0.5 g. Increased growth of body hair in males with SCA also occurred during zinc administration.

Leg ulcers, present in about 75 percent of patients with SCA, have been reported by Serjeant et al.⁴ to respond to oral zinc sulfate. In the aforementioned study by Prasad et al.,³ of the three patients with chronic leg ulcers complete healing was observed in one and some improvement of the ulcers of the two other patients was observed.

Plasma zinc concentrations increased significantly during zinc administration whereas only slight increases in erythrocyte zinc and carbonic anhydrase levels were observed. Plasma ribonuclease activity was decreased, but not significantly.

The finding that zinc therapy caused apparent symptomatic improvement in the

clinical signs of sickle cell disease suggests that zinc supplementation may be beneficial in treating SCA patients. As the authors mention, however, the long-term effects of such high dosages of zinc need to be evaluated and confirmation of these findings is needed. □

1. F. J. Oelshlegel, Jr., G. J. Brewer, A. S. Prasad, C. Knutsen and E. B. Schoomaker: Effect of Zinc on Increasing Oxygen Affinity of Sickle and Normal Red Blood Cells. *Biochem. Biophys. Res. Commun.* 53: 560-566, 1973
2. F. J. Oelshlegel, Jr., G. J. Brewer, C. Knutsen, A. S. Prasad and E. B. Schoomaker: Studies on the Interaction of Zinc with Human Hemoglobin. *Arch. Biochem. Biophys.* 163: 742-748, 1974
3. A. S. Prasad, E. B. Schoomaker, J. Ortega, G. J. Brewer, D. Oberleas and F. J. Oelshlegel, Jr.: Zinc Deficiency in Sickle Cell Disease. *Clin. Chem.* 21: 582-587, 1975
4. G. R. Serjeant, R. E. Galloway and M. C. Guerri: Oral Zinc Sulfate in Sickle-Cell Ulcers. *Lancet* II: 891-892, 1970

CELL-MEDIATED IMMUNITY TO GLIADIN WITHIN THE SMALL-INTESTINAL MUCOSA IN CELIAC DISEASE

Cultures of jejunal biopsy specimens support the hypothesis that local cell-mediated immunity to α -gliadin is responsible for villous atrophy and crypt hyperplasia in celiac disease.

Key Words: celiac disease, cell-mediated immunity, α -gliadin

Celiac disease is characterized by diffuse involvement of the entire small bowel with villous atrophy and obvious crypt hyperplasia which prevent proper absorption.¹ Despite the well recognized positive results afforded by treatment with a diet devoid of wheat products, the etiology of celiac disease remains elusive.² The gliadin moiety of wheat gluten has been shown to be responsible for the symptoms associated

with celiac disease,¹ but the mechanism of its action has only been speculated. Two theories are proposed, one links celiac disease to the absence of a critical enzyme in the jejunum and the other suggests that the jejunum in celiac disease is more susceptible to antigenic reaction to gliaden causing submucosal lesions.^{1,3} Early reports emphasized the importance of humoral immunity in the later mechanism possibly since procedures for determining antibody production were more estab-

lished. Specific circulating antibodies have been repeatedly detected in celiac disease.^{4,5} More recently increased intra-epithelial lymphocyte counts have also been shown to parallel the jejunal mucosa destruction in untreated celiac disease.⁶ This has suggested an additional cell-mediated immune reaction whereby sensitive leukocytes release a substance noxious to the surrounding medium which initiates ultrastructural abnormalities in the jejunal mucosa. Allograft rejection by the small bowel⁷ and *Nippostrongylos brasiliensis* infection⁶ in mice involve both villous atrophy and crypt hyperplasia and are induced by immunological factors known to be of cellular origin. Verification of a similar cell-mediated immunological phenomenon in celiac disease could provide an important link between these conditions and other diseases involving abnormal changes in subepithelial morphology associated with lymphoid cell hyperinfiltration.

To date, little progress has been made to determine the role of cell-mediated immunity in celiac disease using only measurements of peripheral-blood lymphocyte concentrations in response to a gluten challenge. Ferguson et al. recently measured some lymphocyte functions in tissue cultures of jejunal mucosa of patients with celiac disease.⁹ Jejunal biopsy specimens were cultured both with and without α -gliadin antigen. The medium from these cultures was incubated with normal human leukocytes in leukocyte-migration chambers and migration of leukocytes was measured.

No inhibition of migration was seen when gliadin was not present in the culture medium of 43 of 44 controls for the celiac-afflicted patients. When α -gliadin was added to the culture medium, significant migration-inhibition was seen in all six persons having untreated celiac disease. No change in migration was detected, however, in cultures from control patients or from the patients whose celiac disease was being treated by a gluten-free diet.

Ferguson et al. suggested that a lymphokine, which is secreted by the lymphocytes upon specific antigenic stimulation with α -gliadin and is normally a mediator of cellular immunity, in celiac disease acts as an instigator of delayed hypersensitivity. The implications are that humoral and/or other antigenic factors may also be involved.

As this is a preliminary communication, continued effort to link cell-mediated immunity and celiac disease seem to be guaranteed. Such investigations not only aid in elucidating the disease process but also are prerequisites to the development of workable assay methods. □

1. *Gastrointestinal Disease* by M. H. Sleisinger and J. S. Fordtran. Pp. 58-60. W. B. Saunders Co., Philadelphia, 1973
2. *Diseases of the Digestive System* by S. C. Truelove and P. C. Reynell. Pp. 311. Blackwell Scientific Publications, Oxford, England, 1972
3. M. Shiner: Ultrastructural Changes Suggestive of Immune Reactions in the Jejunal Mucosa of Coeliac Children Following Gluten Challenge. *Gut* 14: 1-12, 1973
4. K. G. Kenrick and J. A. Walker-Smith: Immunoglobulins and Dietary Protein Antibodies in Childhood Coeliac Disease. *Gut* 11: 635-640, 1970
5. F. Carswell and R. W. Logan: Plasma β_1 C- β_1 A Globulins and Immunoglobulins in Coeliacs Disease. *Arch. Dis. Child.* 48: 587-589, 1973
6. M. Shiner and D. H. Shmerling: The Immunopathology of Coeliac Disease. *Digestion* 5: 69-88, 1972
7. A. Ferguson and D. M. V. Parrott: Histopathology and Time Course of Rejection of Allografts of Mouse Small Intestine. *Transplantation* 15: 546-554, 1973
8. A. Ferguson and E. E. Jarrett: Hypersensitivity Reactions in the Small Intestine. I. Thymus Dependence of Experimental 'Partial Villous Atrophy. *Gut* 16: 114-117, 1975
9. A. Ferguson, J. P. McClure, T. T. MacDonald and R. J. Holden: Cell-Mediated Immunity to Gliadin within the Small-Intestinal Mucosa in Coeliac Disease. *Lancet* I: 895-897, 1975

HYPERVITAMINOSIS E AND COAGULATION

Coagulopathy has been associated with excess vitamin E ingestion. It is postulated that the observed coagulopathy is a result of the direct interference of vitamin E with vitamin K activity.

Key Words: coagulopathy, hypervitaminosis E, ecchymoses, prothrombin time, hematoma

In recent years few vitamins have received more attention than has vitamin E. This fat soluble vitamin has been prescribed in large doses for a variety of disorders ranging from cardiovascular disease to muscular dystrophy.^{1,2} In addition to prescribed clinical use, the vitamin is widely consumed by the public for less well-founded reasons. The vitamin is readily available since its purchase does not require a prescription. Although it is currently popular to consume massive doses of this vitamin, little work appears to have been done on the effects of excess vitamin E. To date vitamin E is presumed to be essentially nontoxic to animals and humans.¹

The available information on possible toxic effects of vitamin E is somewhat contradictory. Mellette and his colleagues³ report that the addition of large amounts of vitamin E to the diet of rats fed non-irradiated and irradiated beef diets, increased mortality and coagulopathy as evidenced by depressed prothrombin levels. More recently, March and co-workers⁴ also reported that clotting time was lengthened in chicks fed high levels of vitamin E in the diet. In contrast Dymsha and Park⁵ recently reported no adverse effects to rats fed 50 times the normal allowance of vitamin E, and Farell et al.⁶ concluded from their study of men habitually consuming 100 to 800 IU vitamin E per day, that megavitamin E supplements produced no apparent toxic effects as measured by screening of several parameters including coagulation.

Although serious clinical effects of hypervitaminosis E were not reported in

the preceeding studies, Corrigan and Marcus⁷ observed prolonged prothrombin time and ecchymoses in one 55-year-old male patient who was taking warfarin sodium and clofibrate concomitant with self-administration of up to 1200 IU of vitamin E per day. After discontinuation of the warfarin and vitamin E they observed a clearing of the ecchymoses and a reduction in the prothrombin time which was maintained when warfarin treatment was reinstituted.

After the patient's clinical and hematological status stabilized for two months, the investigators challenged the patient with 800 IU per day of vitamin E for 42 days. During this period he continued to receive 2.5 to 5 mg daily of warfarin sodium and 2 g daily of clofibrate.

A progressive increase in prothrombin time was observed throughout the test period. Simultaneously, levels of the vitamin K-dependent coagulation factors declined reaching their lowest point by the 42nd day of vitamin E ingestion. On the 42nd day multiple ecchymoses and a hematoma appeared, at which time vitamin E treatment was discontinued.

One week after vitamin E was discontinued, prothrombin time and levels of the coagulation factors returned to the levels established prior to initiation of vitamin E therapy. With the restoration of normal coagulation, there was concomitant cessation of all clinical evidence of hemorrhage.

The investigators suggested that since warfarin is known to depress levels of vitamin K-dependent coagulation factors, and that clofibrate potentiates the warfarin

effect, a synerism between clofibrate and vitamin E may explain the coagulopathy. Animals treated with clofibrate, however, tend to have low serum levels of vitamin E arguing against a synergistic effect. It is also possible that an interaction between warfarin and vitamin E occurs. Warfarin is partially metabolized by oxidative enzymes in the liver; vitamin E, being an antioxidant, could interfere with the degradation of warfarin. However, this also does not appear to be the case since plasma warfarin levels remained virtually unchanged during the test period. Thus, this data and current knowledge of these drugs does not support the thesis that the vitamin E toxicity was drug induced, but rather that there is a direct interference of vitamin E with vitamin K activity. This suggestion is supported, in part, by the observation that vitamin K-deficient animals treated with vitamin E have further depression of vitamin K-dependent factor levels.

In view of the interesting observations of these investigators and the fact that the recommended intake of vitamin E is greatly exceeded in many situations, the possibility

of adverse responses to excess vitamin E cannot be disregarded. □

1. *The Pharmacological Basis of Therapeutics*. L. S. Goodman and A. Gilman, Editors, fourth edition, pp. 1694-1697. The Macmillan Co., New York, 1970
2. G. M. Berneske, A. R. Butson, E. N. Gauld and D. Levy: Clinical Trial of High Dosage Vitamin E in Human Muscular Dystrophy. *Canad. Med. Assn. J.* 82: 418-421, 1960
3. S. J. Mellette and L. A. Leone: Influence of Age, Sex, Strain of Rat and Fat Soluble Vitamins on Hemorrhagic Syndromes in Rats Fed Irradiated Beef. *Fed. Proc.* 19: 1045-1049, 1960
4. B. E. March, E. Wong, L. Seier, J. Sim and J. Biely: Hypervitaminosis E in the Chick. *J. Nutrition* 103: 371-377, 1973
5. H. A. Dymsha and J. Park: Excess Dietary Vitamin E in Rats. *Fed. Proc.* 34: 912, 1975
6. P. M. Farrell and J. W. Willison: Megavitamin E Supplementation in Man. *Fed. Proc.* 34: 912, 1975
7. J. J. Corrigan, Jr. and F. I. Marcus: Coagulopathy Associated with Vitamin E Ingestion. *J. Am. Med. Assn.* 230: 1300-1301, 1974

ORAL FEEDING VERSUS TOTAL PARENTERAL NUTRITION IN LOW BIRTHWEIGHT INFANTS

There was no significant difference in neonatal mortality and morbidity between infants receiving total parenteral nutrition (TPN) and controls who were fed "humanized" milk by continuous nasogastric drip.

Key Words: total parenteral nutrition, low birth weight infants

The provision of adequate nutrition is a major problem in the management of newborns of low birthweight. Animal studies suggest that neonatal malnutrition can cause permanent retardation of brain development. It is even more imperative to provide adequate nutrition to the small birthweight infant who has a reduced store of nutrients. Cornblath et al.¹ found that intravenous sugar and amino acid solutions did not result in significant weight gain in such infants, possibly because calorie in-

take was limited by the amount of water which could be infused. Higgs et al.³ postulate that if increased amounts of energy could be given parenterally the improved utilization of amino acids and positive nitrogen balance would result in better weight gain.² Wilmore and Dudrick⁴ successfully treated infants by total parenteral administration of protein hydrolysate, electrolytes, hypertonic glucose and vitamins using an indwelling venous catheter (TPN).

Recently TPN was compared with continuous nasogastric drip (oral) in the treat-

ment of low birthweight infants.¹ A 10 percent invert sugar solution was begun two hours after birth, first by scalp vein infusion and thereafter at 6-12 hours by umbilical catheter. After 24 hours oral or parenteral nutrition was commenced. Two groups of 43 infants were randomly selected to receive either oral feeding or TPN. The oral group was fed full-strength "humanized" milk. The milk infused the first three days was supplemented intravenously with 10 percent sugar. Intakes of 166 kcal and 3.2 g protein per kilogram of body weight per day were reached by the fifth day.

The TPN group received no oral feeding for ten days, but received parenteral fluids (casein hydrolysate, fat emulsion and dextrose) by continuous umbilical arterial infusion. By the fourth day, maximum intakes of 136 kcal per kilogram of body weight and 3.2 g protein were reached. The entire system, except the arterial catheter, was changed daily until day 10 at which time oral full-strength "humanized" milk feeding was initiated. Both groups received routine oral milk and nursery care for the next 14 to 28 days.

The mortality rate for both treatments was high with pneumonia, septicemia, apnea and enteritis being the most common form of morbidity. In the oral group mortality was 14 percent, while in the TPN group mortality was 20 percent. The added risk of infection from the indwelling catheter with TPN⁵ apparently was not a problem with the short period of parenteral nutrition used in this study.

Though results of biochemical analysis of serum and urine were not included, serum sodium potassium chloride, calcium phosphate, blood urea, serum osmolarity, total protein, albumin, globulin and SGOT were all reported to be within normal limits. Serum cholesterol and plasma amino acid levels were significantly increased during TPN and returned to normal when discontinued. Ghadmi et al.⁶ also reported high levels of amino acids and particularly of methionine, but no harmful effects of

abnormally high levels of amino acids or unbalanced amino acid ratios caused by administration of protein hydrolysates have been proven.⁶

The authors found no significant difference in weight gain between the two groups. Unfortunately, a strict comparison is not possible since the infants were not given comparable diets. The diet of the oral group contained more calories, carbohydrate, fat and ash than that of the TPN group. The fact that the TPN caused the same weight gain although it provided fewer calories may indicate more efficient food utilization with TPN.

Although there is an urgent need for making TPN benefits available to low birthweight infants, ideal parenteral solutions may not be available. Nutritional needs may be significantly different when food is given parenterally instead of orally; and the possible detrimental effects of the altered blood amino acid pattern and elevated lipid and cholesterol levels resulting from administering such solutions as used in this study should be determined. □

1. M. Cornblath, A. E. Forbes, R. S. Pildes and J. Greenard: A Controlled Study of Effect of Early Fluid Administration on Survival of Low Birth Weight Infants. *J. Pediat.* 69: 911-912, 1966
2. M. E. Shils: Guidelines for Total Parenteral Nutrition. *J. Am. Med. Assn.* 220: 1721-1729, 1972
3. S. C. Higgs, A. F. Malan and H. De V. Heese: A Comparison of Oral Feeding and Total Parenteral Nutrition in Infants of Very Low Birthweight. *S. Afr. Med. J.* 48: 2169-2173, 1974
4. D. W. Wilmore and S. J. Dudrick: Growth and Development of an Infant Receiving all Nutrients Exclusively by Vein. *J. Am. Med. Assn.* 203: 860-864, 1968
5. D. W. Wilmore and S. J. Dudrick: Safe Long-Term Venous Catheterization. *Arch. Surg.* 8: 256-258, 1969
6. H. Ghadimi, F. Abaci, S. Kumar and M. Rathi: Biochemical Aspects of Intravenous Alimentation. *Pediatrics* 48: 955-965, 1971

*The Journal of
Laboratory and Clinical
Medicine*

October, 1917

Volume III, No. 1, Pages 40-49

THE PREVENTION OF SIMPLE GOITER IN MAN

A Survey of the Incidence and Types of Thyroid
Enlargements in the Schoolgirls of Akron (Ohio),
from the 5th to the 12th Grades,
Inclusive—The Plan of Prevention Proposed.

By David Marine, M.D., and
O. P. Kimball, B.S., Cleveland, Ohio

Simple goiter in animals is probably the easiest of all known diseases to prevent. Simple goiter includes all the thyroid enlargements seen in the lower animals and those thyroid enlargements seen in man, except cases properly classified as exophthalmic goiter. Many cases with simple goiter later develop exophthalmic goiter. In brief, simple goiter includes all those thyroid enlargements formerly classified as endemic, epidemic and sporadic. The periods when it most frequently develops are (1) fetal, (2) adolescent, and (3) during pregnancy.

• • • •

12 NOV 1975

It will not be possible to review all the experimental data on which the assertion, that simple goiter in animals is an easily preventable disease, is based. Certain of the more important facts bearing on the subject will be summarized as an introduction to the discussion of the means proposed to attempt the prevention of simple goiter in man.

1. The developmental stage of all goiters is characterized by an increased blood flow, an increase in the size and number of epithelial cells, a decrease in the stainable colloid of the follicular spaces and a marked absolute decrease in the iodine content. The decrease in iodine preceeds the cellular changes.

2. Similar thyroid changes (compensatory hyperplasia) invariably occur in the remaining portion of the gland when a sufficient portion of the entire gland is removed. The amount of gland it is necessary to remove in order to cause compensatory hyperplasia varies somewhat with the species of animal, definitely with the age, the diet, and the presence of iodine.

3. The administration of exceedingly small amounts of any salt of iodine thus far tried in any manner completely protects the remaining thyroid against compensatory hyperplasia, even after the removal of three-fourths of the normal gland in cats, dogs, rabbits and rats, fowls and pigeons.

4. We have repeatedly found that a milligram of iodine given at weekly intervals is sufficient to prevent thyroid enlargement, although other pups of the same litter, living in the same kennel, and eating the same food, regularly developed goiter.

5. The thyroid gland has an extraordinary affinity for iodine, as can readily be shown by perfusion experiments *in vitro* or by injecting small amounts—5 to 20 mg. KI.—into the circulation. Experimentally then the proof is sufficiently complete to demonstrate the underlying principles of goiter prevention in animals and the ease with which they can be applied. From the practical standpoint, the first instance of preventing goiter on a large scale was accidental and in connection with the sheep raising industry of Michigan. Prior to the discovery of salt deposits around the Great Lakes, the future of the industry seemed hopeless, but with the development of the salt industry and its use by the sheep growers, goiter rapidly decreased. The salt contains appreciable quantities of both bromine and iodine and in places these elements are extracted on a commercial scale. The second instance of goiter prevention on a large scale was in brook trout. Some years ago the development of

goiter in artificially raised members of the salmon family became alarming and many plants were abandoned on account of the disease. After considerable work, which led to the conclusion that the disease was simple goiter, we were able to completely prevent the disease in several hatcheries by the use of very small amounts of tincture of iodine added to the water.

• • • •

In spite of this knowledge of the ease and simplicity of goiter prevention in the lower animals, we know of no instance where the attempt has been made to systematically prevent or control the disease in children in large communities, especially those of the Great Lakes Basin, where goiter is so prevalent. Locally, we have been carrying out preventive treatment for the past six years at the Lakeside Hospital Medical Dispensary and have urged local physicians to do so in their private practices. A great deal has been accomplished in this way, but as it is a public health matter the most practical and economic method would be to utilize the Public School System and the Board of Health. When the Medical Inspection of Schools is more or less independent of the Board of Health, it would be carried out through the Medical Director of Schools. This year it has been possible to begin such work on a large scale in the city of Akron, through the cooperation of the Superintendent of Schools, the Board of Education, and the County Medical Society.

It was decided for the present to limit the prophylactic work to the girl pupils, since adolescence is the most important goiter developing period and since at this period it occurs about six times more frequently in girls than in boys.

The plan now in operation was arranged from the standpoint of simplicity, practicability, economy, and the possible scientific value of the data obtained. Changes will doubtless be made as the work progresses. First a census of the condition of the thyroid gland was taken of all girls between the 5th and 12th grades.

We have, therefore, arbitrarily selected to use 2 gm. sodium iodide, given in 0.2 gm. doses each school day, for each pupil in the 5th, 6th, 7th, and 8th grades; and 4 gm. given in 0.4 gm. doses each school day for each pupil in the 9th, 10th, 11th, and 12th grades. These amounts will be given twice annually about the first of May and December, at the schools by the teachers or nurses.

• • • •

Analysis of the Thyroid Examinations. —Three thousand eight hundred and seventy-two girls of the 5th, 6th, 7th, 8th, 9th, 10th, 11th and 12th grades were examined and the general result is given in the following tabulation.

o o o o

TABLE III.

CONDITION OF THYROID ARRANGED ACCORDING TO AGES

Age	10-12		12-14		14-16		16-18		18-20	
	No. of Cases	%	No. of Cases	%	No. of Cases	%	No. of Cases	%	No. of Cases	%
Normal	530	56.08	521	41.32	460	40.35	156	34.44	21	28.77
Slightly Enlarged	394	41.69	680	53.92	578	50.70	235	51.88	44	60.27
Moderately Enlarged	21	2.22	59	4.68	98	8.6	60	13.24	9	10.96
Markedly Enlarged			1	0.08	4	0.35	2	0.44		
Totals	945	24.41*	1261	32.56	1140	29.44	453	11.70	73	1.89
Adenomas	2	0.01**	11	0.52	18	0.84	8	0.39		

* Percentage of total pupils.

** Percentage of total enlarged thyroids.

o o o o

SUMMARY

In a complete census of the condition of the thyroid gland in the girls from the 5th to 12th grades of the school population of a large community in the Great Lakes goiter district, it was found that 1688, or 43.59 per cent, had normal thyroids; 2184, or 56.41 per cent, had enlarged thyroids; and 594, or 13.4 per cent, had well-defined, persistent thyroglossal stalks. The community lies near the southern edge of the goiter district and it is suggested that communities near the lakes would show a higher incidence. The method of prophylaxis proposed is in operation.

URINARY PHENYLALANINE METABOLITES IN PREWEANLING RATS TREATED WITH *p*-CHLOROPHENYLALANINE AND PHENYLALANINE

The p-chlorophenylalanine plus phenylalanine-treated rat is a widely accepted model for generation of a PKU-like syndrome in rats. Examination of the urinary metabolites of phenylalanine in preweanling rats showed that a regimen sufficient to produce the pathological state did not result in increased levels of the classical aromatic acidic metabolites, phenylpyruvate and phenyllactate.

Key Words: PKU, *p*-chlorophenylalanine, phenylalanine, phenylpyruvate, phenyllactate

Phenylketonuria (PKU) was described in 1934 and is now known to be the most prevalent of the aminoacidurias. In the classical syndrome, there is a complete absence of phenylalanine hydroxylase in the liver. Since this is the first enzyme on the main catabolic pathway of phenylalanine, its deletion leads to high blood levels of the amino acid and its alternate metabolites, particularly phenylpyruvate. Unless the dietary intake of phenylalanine is reduced to a level just sufficient for protein synthesis, mental retardation ensues.

Despite the substantial research on this problem, the biochemical mechanism by which phenylalanine or its metabolites retard brain development has not been established. In recent years, the concept that the phenylketo metabolites are the key substances received increasing support through the demonstration that phenylpyruvate is capable of inhibiting a large number of enzymes spanning a variety of important pathways. In addition, variants of the disease occur where only plasma phenylalanine is elevated, but phenylpyruvate remains normal and brain development is not retarded.

Demonstration of a pathogenetic mechanism often requires an animal model in which the disease can be mimicked, enabling biochemical changes to be assessed concurrent with the onset and development

of the pathology. The discovery that *p*-chlorophenylalanine (*p*CP) is a specific inhibitor of phenylalanine hydroxylase in vivo and in vitro stimulated its use along with high phenylalanine intakes as such a model. Prolonged application of this regimen to preweanling rats does cause the appearance of behavioral and neuropathological abnormalities similar to those seen in clinical PKU.^{1,2}

A logical next step would be the determination of the array of urinary phenylalanine metabolites which appear in the treated rat and the comparison of the pattern to that which occurs in humans. Such a study has been recently completed by Rowe et al.³ Uniformly labeled ¹⁴C-L-phenylalanine was injected into 18 to 22 day old rats and urine was collected for the following six hours. Simultaneous administration of an osmotic diuretic (urea) and use of an electrified grid to induce micturition assured maximum recovery of urinary metabolites. Separation, identification and quantification of the metabolites was accomplished by a combination of paper, thin-layer and gas chromatography and by use of a GC-mass spectrometer. Six groups of rats were employed. Group 1, the control, received only the tracer dose of phenylalanine. Group 2 was injected with 60 mg *p*CP per kilogram of body weight 24 hours before the tracer. Group 3 was not given any *p*CP but was given 340 mg per kilogram of body weight

of cold phenylalanine simultaneously with the tracer. Group 4 was given two injections of a solution which contained both *p*CP and cold phenylalanine, one 24 hours before the label and one with the labeled phenylalanine. Group 5 (heavily loaded) was treated identically to Group 4 but given an additional 4 g phenylalanine per kilogram of body weight from between two and six hours before the label. Group 6 (chronic) consisted of a single litter treated with the standard dose of *p*CP plus phenylalanine from day 10 to day 21 and then treated the same as Group 4 on day 22.

When the urine samples were fractionated by means of paper chromatography, the radioactivity was, in all cases, approximately equally divided between the amino acid fraction and an acidic metabolite fraction. The amino acid fraction consisted of tyrosine and phenylalanine while the acidic metabolite fraction was predominantly (75 percent) hippuric acid and phenaceturic acid (N-phenylacetylglycine). The administration of cold phenylalanine with or without *p*CP caused an increase in the overall excretion of acidic metabolites but did not change the ratio.

The acidic metabolite fraction was separated upon a gas chromatograph system after silylation and small amounts of phenyllactic acid, *p*-hydroxybenzoic acid, *m*-hydroxyphenylacetic acid, *p*-hydroxyphenylacetic acid, *p*-hydroxy-mandelic acid, and *p*-hydroxyphenyllactic acid were found to be present as minor constituents. *p*-Chlorophenaceturic acid was the only *p*CP metabolite found in the urine of the treated animals. Phenylpyruvic acid was notably absent from the urine of animals from Groups 1 through 4 and Group 6. It was present in Group 5 (*p*CP and heavily loaded with phenylalanine) in addition to a substantial increase in phenyllactic acid. A gas chromatograph pattern of the urine extracts from Group 5 was found to have a close resemblance to

that obtained from two mature institutionalized PKU subjects.

The authors emphasize several points in their discussion. The data showed that the major urinary metabolites of phenylalanine in young rats are the conjugated derivatives, hippuric acid and phenaceturic acid. Moreover, this was also true in those animals (Group 6) which had received a standard dose of *p*CP and phenylalanine for 12 days, a regimen which elevates serum phenylalanine and which elicits PKU-like behavioral and pathological changes. Thus, while the unconjugated metabolites phenylpyruvate and phenyllactate can be detected in *p*CP-treated rats after massive loading with phenylalanine, they do not appear to be necessary to the production of the neurological damage in the model.

These results keep alive a very exciting controversy regarding the biochemical mechanisms responsible for the neurological damage in PKU. They also demonstrate the need to examine any model system with great care. Is the *p*CP plus phenylalanine model valid for the generation of PKU in the rat when the classical metabolites of the human disease are absent? Or should this be taken as evidence that the phenylketo derivatives themselves are not the primary agents responsible for the damage? The resolution of these questions should be useful and interesting. □

1. A. E. Andersen and G. Guroff: Enduring Behavioral Changes in Rats with Experimental Phenylketonuria. *Proc. Nat. Acad. Sci. USA* 69: 863-867, 1972
2. A. E. Andersen, V. D. Rowe and G. Guroff: The Enduring Behavioral Changes in Rats with Experimental Phenylketonuria. *Proc. Nat. Acad. Sci. USA* 71: 21-25, 1974
3. V. D. Rowe, H. M. Fales, J. J. Pisano, A. E. Andersen and G. Guroff: Urinary Metabolites of Phenylalanine in the Prewanling Rat Treated with *p*-Chlorophenylalanine and Phenylalanine. *Biochem. Med.* 12: 123-136, 1975

GROWTH AND CEREBRAL LIPID COMPOSITION DURING MATURATION OF RATS FED VARYING LEVELS OF LINOLEATE AND LINOLENATE

Rats were fed diets containing 3.0, 0.75 or 0.07 percent of the calories as essential fatty acids (EFA) for three generations. In general, growth and fatty acid composition were very similar at the two higher intakes of EFA. The pattern of linoleate series fatty acids in cerebral phosphatidylethanolamine was very similar with all three groups. The increase in linolenate series fatty acids depended on the level of dietary linolenate, was much slower and continued to increase with age.

Key Words: essential fatty acid (EFA), linoleate, linolenate, phospholipids, cerebrum, arachidonate

The extent to which variations in dietary fatty acid composition might alter brain fatty acid composition is an especially important question, in view of the possibility that changes in brain fatty acid composition might alter brain function.¹ Considerable study has been given to lipid and fatty acid composition of brain in rats fed diets deficient in essential fatty acids (EFA).² That changes in function do occur is indicated by reports of alterations in brain levels of enzymes such as 5'-nucleotidase and Na⁺,K⁺-ATPase in EFA deficiency.^{3,4}

In the majority of the studies of EFA deficiency, the usual experimental procedure has been to start the EFA-deficient diet late in pregnancy. Under these circumstances, however, the extent of change in brain EFA in the offspring has been greatly affected by the reserve of EFA available in the dams during lactation. Also, with this type of experiment, it is not possible to determine how brain development might be affected by EFA depletion during the intrauterine phase since the EFA reserves of the dams were usually high. Furthermore, little attention has been paid to the proportions of linoleate and linolenate supplied at any given level of dietary fat. In view of evidence for possible unique functions of linolenate, especially in vision,⁵ the ratio of linoleate to linolenate in the dietary fat cannot be ignored.

Ailing and co-workers⁶⁻⁹ conducted long-term studies in which diets varying in EFA level, but with a fixed ratio of linoleate to linolenate, were fed to rats for three generations, with body compositions and lipid analysis done on rats of the third generation. Under these conditions, the effect of a given dietary EFA composition would be exerted during the intrauterine, suckling and postweaning periods.

The diets contained 100 g fat per kilogram of diet, but the proportions of EFA were varied so that 3.0, 0.75 or 0.07 percent of the dietary calories were from EFA. This was made possible by combining hydrogenated lard, sunflower oil or linseed oil such that the ratio of linoleate (18:2N6) to linolenate (18:3N3) was 4:1 in all diets except the low-fat (0.07) diet. The 0.07 level was obtained with the hydrogenated lard alone as the fat source. This diet, when analyzed after mixing, contained 200 mg linoleate and 30 mg linolenate, for a ratio of 10:1. Some EFA were supplied by corn starch, which was the major dietary carbohydrate (178 g per kilogram or 16 percent of the calories) and by a fish concentrate (defatted), which was the dietary protein source, fed as 16 percent of the calories. Other components were identical in all diets.

Female rats were raised on diets supplying 3.0, 0.75 or 0.07 percent of the calories as EFA and 16 percent of the calories as protein (HP 3.0, HP 0.75 and HP 0.07).

The females were bred when they were 90 days old. A few rats, previously fed the HP 0.75 diet, were also fed the HP 0.07 diet three weeks before mating. Other females were transferred at mating from the HP 3.0 and HP 0.75 diets to a low protein diet (LP) supplying 8.0 percent of the calories as protein and containing the same EFA levels, i.e., LP 3.0 and LP 0.75 diets. At birth, the litters were reduced to six rats each. The young remained with the dams until weaning at 21 days.

The largest litters occurred with the HP 3.0 diet. No deaths occurred in this group in the suckling period. Two deaths occurred with the HP 0.75 and LP 3.0 groups in this period. In contrast, in the LP 0.75 group, four rats out of 48 (eight litters of six each) died.

The concentration of linoleate in milk triglycerides was directly proportional to dietary linoleate and appeared to stay constant during lactation, on the basis of samples taken at four and 14 days. The triglyceride concentration varied between 190 and 257 μ moles per milliliter. The phospholipid (PL) concentration, which was 38 to 50 μ moles per milliliter, supplied up to 10 percent of the EFA in the milk samples. The contribution of PL to the milk EFA concentration was higher in samples from rats fed the low EFA diets, an observation which agrees with other data.¹⁰

Between 25 and 60 days of age, the young rats fed the HP 3.0 diet were heavier than those fed the HP 0.75 diet, but from 60 to 90 days, the weights for these two groups were similar. At all ages, the group fed the HP 0.07 diet were the smallest, ca. 267 g for the males, in comparison with ca. 293 g for the HP 3.0 and HP 0.75 diets. The male rats fed the LP 3.0 or LP 0.75 diets weighed only 154 g at 90 days of age. Skin lesions appeared on the tail in rats from the HP 0.07 group, but later disappeared. The disappearance of the tail lesions did not result from a change in humidity since the humidity of the animal room was constant throughout the experiment.

Analysis of body composition showed that the proportion of total body fat was similar in the rats fed the HP 3.0 and HP 0.07 diets, despite the difference in body weight. The rats fed the HP 0.75 diet had a higher proportion of body fat than rats fed the HP 3.0 diet. The differences in body fat were greatest in female rats at 120 days of age. For example, the females fed the HP 0.75 diet weighed 225 g and contained 13.5 g fat per 100 g body weight. Those fed the HP 3.0 diet weighed 223 g and contained 10.0 g fat per 100 g body weight. The concentrations of nitrogen, potassium and ash did not differ. As expected, higher levels of linoleate and arachidonate occurred in the body fat of rats fed the diets richer in EFA.

Samples of the gastrocnemius and quadriceps muscles were analyzed to determine the effect of EFA intake on fatty acid composition of skeletal muscle lecithin. Muscle was selected since it is the largest body tissue component. Also, there is some controversy on whether EFA deficiency affects muscle calcium permeability.^{11,12} Rats fed the HP 3.0 and HP 0.75 diet had higher concentrations of muscle phospholipids than did rats fed the HP 0.07 diet. The proportions of polyenoic fatty acids of the linoleate series in muscle lecithin depended upon the dietary EFA level. Yet after the rats were 45 days of age, the level of linoleate series fatty acids in the HP 0.75 group approached that of the HP 3.0 group, despite the differences in dietary EFA intake.

The sum of the linolenate series fatty acids varied with the EFA level of the diet except with the HP 0.07 rats, in which the ratio of linoleate series to linolenate series fatty acids was 10:1. In the HP 3.0 and HP 0.75 rats, the ratio of linoleate to linolenate series fatty acids was the same, but in the HP 0.07 rats, this ratio was increased.

Cerebral weights were lower in rats fed the HP 0.07 diet than with the HP 3.0 and HP 0.75 groups, but the ratio of cerebral weight to body weight was similar for all diets. The concentration of total phospho-

lipid (μ moles per gram of wet tissue) was the same in all groups at all ages tested. However, the cerebroside concentration was somewhat lower in the rats fed the HP 0.07 diets, for the first 30 days of life. Yet at 45 and 120 days the cerebroside concentration in the HP 0.07 group was the same as in the HP 3.0 and HP 0.75 groups. The decreased cerebroside concentration before 30 days in the HP 0.07 group may indicate altered myelin formation since cerebroside are important constituents of myelin. Although there is an apparent recovery at 120 days, this provides no assurance that cerebral function is still normal in these rats. The cerebrum was analyzed, rather than the entire brain since they considered^{1,3} that any changes in brain maturation could be evaluated better if they were detected in specific regions of the brain.

The accumulation of linoleate series fatty acids in the cerebrum (μ moles per cerebrum), was generally similar at all ages with all diets, despite the differences in linoleate intakes produced by the different diets. The accumulation of linolenate series fatty acids, however, depended upon the amount in the diet. The rats fed the HP 3.0 diet had a higher concentration of linolenate series fatty acids (μ moles per cerebrum) than did rats fed the HP 0.75 diet, although the dietary ratio of linoleate to linolenate was the same. The accumulation of linolenate series fatty acids was slower than that of the linoleate series fatty acids up to 24 days. After 24 days, the linoleate series fatty acids increased only slightly. However, the linolenate series fatty acids continued to increase, although less rapidly, in the HP 3.0 and HP 0.75 groups. The accumulation was very slow with the HP 0.07 groups, however, and almost no additional deposition occurred between 30 and 45 days.

When the cerebral phosphatidylethanolamine fraction was analyzed, arachidonate was found to be the major polyunsaturated fatty acid of the linoleate series. The proportion of arachidonate was similar in all groups at all ages. Arachidonate increased up to 20 to 25 percent of the fatty

acids in the first ten days, then declined to 15 to 20 percent. Docosahexaenoate (22:6N3) was the only major fatty acid of the linolenate series present. Its proportion increased with age in all groups. The sum of 22:6N3 and 22:5N6 (docosapentaenoic acid of the linoleate series) was less at birth with the HP 0.07 groups than with the HP 3.0 group, but increased with age up to 100 days, so that it equaled the values for the HP 3.0 and HP 0.75 groups. The proportion of these fatty acids in the HP 3.0 and HP 0.75 groups remained quite constant with age.

The lower levels of linolenate series fatty acids with the HP 0.07 presumably resulted from the fact that the ratio of linoleate to linolenate was 10:1 in this group, rather than 4:1 as with the HP 3.0 and HP 0.75 groups. However, similar results were obtained with a group fed an HP 0.07 diet, in which the ratio of linoleate to linolenate was 4:1. Thus, the slower accumulation of linolenate-series fatty acids in the HP 0.07 groups could not be attributed simply to the lower intake of linolenate, relative to linoleate, but must reflect some other change resulting from the very low total EFA intake.

The most striking results of these experiments with identical diets, except for the level of EFA, showed the following: (a) the relative constancy of cerebral levels of fatty acids of the linoleate series, despite large differences in dietary intake; (b) the rapidity of accumulation of linoleate series fatty acids; and (c) the slower accumulation of fatty acids of the linolenate series and its dependence on the absolute amount of linolenate in the diet. Both linoleate and linolenate can be oxidized rapidly.¹⁵ At very low linolenate intakes, this rapid oxidation may leave little for the slower process of docosahexaenoate (C22:6N3) formation. With higher linolenate intake, sufficient linolenate may still be available for 22:6N3 formation.

In these experiments more 22:6N3 was accumulated than 22:5N6 in cerebral phosphatidylethanolamine, except at the very low linolenate intakes. Even then, the

proportion of 22:6N3 persistently increased. Whether this pattern of 22:6N3 accumulation indicates a functional requirement for 22:6N3, rather than 22:5N6, is still a major unsolved question.¹⁴ □

1. Present Knowledge of the Relationship of Nutrition to Brain Development and Behavior. *Nutrition Reviews* 31: 242-246, 1973
2. Rat Brain Fatty Acids in Essential Fatty Acid Deficiency. *Nutrition Reviews* 30: 18-21, 1972
3. J. Bernsohn and F. J. Spitz: Linoleic- and Linolenic Acid Dependency of Some Brain Membrane-Bound Enzymes after Lipid Deprivation in Rats. *Biochem. Biophys. Res. Commun.* 57: 293-298, 1974.
4. G. Y. Sun and A. Y. Sun: Synaptosomal Plasma Membranes: Acyl Group Composition of Phosphoglycerides and (Na⁺+K⁺)-ATPase Activity during Fatty Acid Deficiency. *J. Neurochem.* 22: 15-18, 1974
5. Essential Fatty Acid Deficiency and the Photoreceptors of Rat Retina. *Nutrition Reviews* 32: 342-345, 1974
6. C. Alling, Å. Bruce, I. Karlsson and L. Svennerholm: The Effect of Different Dietary Levels of Essential Fatty Acids on Growth of the Rat. *Nutrition Metab.* 16: 38-50, 1974
7. C. Alling, Å. Bruce, I. Karlsson and L. Svennerholm: The Effect of Different Dietary Levels of Essential Fatty Acids on Body Composition of the Rat. *Nutrition Metab.* 16: 181-191, 1974
8. C. Alling, Å. Bruce, I. Karlsson and L. Svennerholm: Effect of Different Dietary Levels of Essential Fatty Acids on the Fatty Acid Composition of Lecithin in Rat Skeletal Muscle. *Nutrition Metab.* 16: 249-259, 1974
9. C. Alling, Å. Bruce, I. Karlsson and L. Svennerholm: The Effect of Different Dietary Levels of Essential Fatty Acids on Lipids of Rat Cerebrum during Maturation. *J. Neurochem.* 23: 1263-1270, 1974
10. C. Galli and C. Spagnuolo: Essential Fatty Acids in Maternal Diet and in Rat Milk Phospholipids. *Lipids* 9: 1030-1032, 1974
11. D. Seiler and W. Hasselbach: Essential Fatty Acid Deficiency and the Activity of the Sarcoplasmic Calcium Pump. *Europ. J. Biochem.* 21: 385-387, 1971
12. B. P. Yu, F. D. DeMartinis and E. J. Masoro: Relation of Lipid Structure of Sarcotubular Vesicles to Ca⁺⁺ Transport Activity. *J. Lipid Res.* 9: 492-500, 1968
13. C. Alling and I. Karlsson: Changes in Lipid Concentrations and Fatty Acid Compositions in Rat Cerebrum during Maturation. *J. Neurochem.* 21: 1051-1057, 1973
14. A. J. Sinclair and M. A. Crawford: The Accumulation of Arachidonate and Docosahexaenoate in the Developing Rat Brain. *J. Neurochem.* 19: 1753-1758, 1972
15. G. A. Dhopeswarkar and C. Subramanian: Metabolism of Linolenic Acid in the Developing Brain. 1. Incorporation of Radioactivity from 1-¹⁴C-Linolenic Acid into Brain Fatty Acids. *Lipids* 10: 238-241, 1975

MECHANISMS IN YOUNG CROCODILIANS GREATLY MODIFYING THE PERSISTENCE OF ADMINISTERED AMINO ACIDS

An explanation is sought in the synthesis of polypeptide or protein for a sharp reduction in the persistence in the blood plasma of administered amino acids when other amino acids are given, even if several essential amino acids are not provided.

Key Words: Caiman, alligator, protein, amino acids, methionine, intestine, digestion, absorption, transport, insulin, cycloheximide, catabolism, growth

Organisms range widely in the proportions in which they make anabolic and catabolic use of administered amino acids, even disregarding the phenomenon of adulthood.

Studies by Coulson and Herbert^{1,2} bring out an informative extreme for Caimans (*Caiman crocodilus crocodilus*) in their second half-year of life. Alligator mississippiensis was so similar that this species could be used interchangeably. An earlier study persuaded Coulson and his associates that the catabolic rates for various injected

amino acids or mixtures of amino acids are so low that accelerated disappearance could be equated with accelerated protein synthesis.

These animals increase their body weight about 50-fold in their first year of life, while human infants increase their weight only three- or four-fold. They may consume quantities of fish so huge that the authors did not consider it unphysiologic to force into their stomachs the equivalent of one-twentieth (or even one-tenth) of their body weight in the form of lean fish muscle.² During the ensuing period of absorption, the total amount of catabolically derived energy available to these cold-blooded animals at 28°C could, if all were available for absorption, provide only a minor fraction of an ATP molecule for each molecule of amino acid absorbed.³

Absorption must therefore be carried out at very low energy costs under the unusual prevailing conditions. One thinks of the possibility that unusual proportions of amino acids might be absorbed in peptide form, or that unusually large quantities of hydrolytic products might pass between the epithelial cells. Amino acid analogs designed to escape specifically mediated transport are known to be absorbed in the intact rat by a relatively slow route apparently not subject to saturation.⁴ Such routes may become conspicuous at 28°C, at high protein contents of the gut and in these specialized animals. The present results nevertheless counsel renewed caution about accepting hypotheses for amino acid transport systems in warm-blooded animals calling for the cleavage of at least one and of as many as three ATP molecules each time an amino acid crosses a cellular membrane.

In most of the experiments the reptiles weighed about 600 g. They were fasted five days, an interval considered necessary to obtain fasting amino acid levels of the plasma at 28°C. Single amino acids were injected intraperitoneally in 0.3 M neutral solution in amounts ranging from 0.32 to 3.5 mmoles per kilogram. A mixture of 20 amino acids (partly in solution and partly

in suspension) was injected, containing half as much of each amino acid as was injected alone. This mixture imitated the composition of fish muscle, except for partial replacement of glutamic and aspartic acids by their amides, and totalled 21 mmoles per kilogram of body weight.

Two incomplete mixtures were made by simply omitting three amino acids, in one case lysine, valine and isoleucine, in the other case methionine, threonine and phenylalanine. Plasma was prepared from heparinized blood collected periodically from the tip of the tail. Amino acids were estimated by an automated amino acid analyzer.

When the amino acids were given singly, their half-lives in plasma ranged from one hour (serine) to 140 hours (methionine). When they were administered instead in the complete mixture, they disappeared at much more uniform rates (half-lives, five to ten hours, mean 7.9 hours), and for methionine, valine, isoleucine, phenylalanine, histidine and threonine, 1.5 to 3.6 times as fast as when given alone. The removal from the plasma of methionine, valine or isoleucine, each supplied alone, was also accelerated by the administration of the complete mixture after a 24-hour delay. The removal of citrulline or norleucine was not accelerated under these conditions, perhaps because these "control" amino acids do not enter protein synthesis.

The authors attribute these accelerations of the depletion of the high plasma levels of about nine amino acids in the mixture to the opening of an alternate route for their assimilation, beyond their ordinarily slow catabolism. This alternate route is presumed to be protein synthesis. The proposal of Elwyn, Parikh and Shoemaker⁵ that incoming amino acids are provisionally converted into liver proteins in the dog was cited for comparison. Nevertheless, the removal of methionine proved to be accelerated about as much when three essential amino acids, in the two different combinations already listed, were omitted from the mixture. This result leads Coulson and Herbert to reason that an incomplete

mixture of amino acids must be converted into a polypeptide form for holding the available amino acids — although it is not clear where the high-energy phosphate is to arise for this provisional synthesis at high protein intakes.

The administration of insulin 24 hours in advance accelerated the removal from the plasma of the total of the exogenous amino acids, whether supplied in the complete mixture or in one of the incomplete mixtures. In contrast, cycloheximide administration slowed their removal, presumably by inhibiting protein synthesis. Previous work led the authors to conclude that insulin acts to lower endogenous amino acid levels by stimulating protein synthesis, and they propose the same action for the present experiments.

We face here the apparent formation of a large hidden reservoir of exogenous amino acids or their metabolic products, for which any explanation may seem for the present preposterous. This reservoir is large enough under the selected conditions, so that its discovery and description should not be so difficult, however, as to justify our accepting any explanation in more than a provisional way. The circumstance that the inclusion of certain amino acids (by no means any or all) accelerates the consumption of certain others (by no means all) allows one to think of interactions for catabolism, for example, partial corrections of depletions of needed co-factors; but the authors argue that accelerated catabolism does not provide an adequate explanation. Apparently the "missing" amino acids are not present as such within the cells of various tissues; "free essential" amino acids are not abundant in liver, kidney, muscle, heart or brain after administering the complete mixture. The totals for "non-essential" amino acids in muscle and kidney seem rather high, however; but in the absence of the corresponding fasting levels, the increments cannot be calculated. Furthermore these tissue levels are decreased, not increased, by insulin administration.

The explanation preferred by the authors is reflected in the running title of their 1974 paper "protein synthesis from incomplete mixtures" (and in the full title, "evidence for polypeptide synthesis...").

In the meantime a number of interesting approaches appear to be available for discovering the detailed nature of the specialization permitting these species to use huge amounts of food protein for growth, with apparently minimal stimulation of their catabolism. An exploration of their endocrine responses seems promising; for example, what are the detailed metabolic effects of insulin, and to what degree is insulin secretion stimulated by leucine and other amino acids?

Herbert and Coulson² show that the peaks of the plasma amino acids come much later (about 60 hours) after feeding 7.5 g fish muscle per kilogram of body weight, than after feeding casein (about 36 hours) or gelatin (about 22 hours). In the latter case the plasma of the fed animals showed a composition in free amino acids resembling that of gelatin, whereas the peak compositions of plasma in the other two cases was quite unlike that of the protein fed, and included high levels of alanine, glutamine and glycine. Zein and several other vegetable proteins appeared on feeding to be excreted intact in the feces.

The rate of absorption of amino acids from the crocodilian gut did not depend on the presence of an optimal ratio of free amino acids, since gelatin was rapidly digested and absorbed, apparently without dilution from other protein sources. Nevertheless, each amino acid fed alone was absorbed at a different rate, whereas when these same amino acids were fed as protein, all amino acids liberated by digestion were absorbed much more rapidly and at the same percentage rate.³ This behavior suggests similarities between the removal of amino acids from the gut and from the plasma, and leads the authors to conclude that the transport of an amino acid presented alone is an "unnatural" event, and

that the large differences in rates of absorption of single amino acids are a physiological artifact. The authors are led to suggest here also a possible intermediate synthesis of amino acids into protein during absorption, although energetic limitations appear to be encountered here also.

Further information on amino acid assimilation in these organisms will be awaited with great interest. □

-
1. R. A. Coulson and J. D. Herbert: Evidence for Polypeptide Synthesis in the Caiman from Mixtures Deficient in Essential Amino Acids. *J. Nutrition* 104: 1396-1406, 1974
 2. J. D. Herbert and R. A. Coulson: Free Amino Acids in Crocodilians Fed Proteins of Different Biological Value. *J. Nutrition* 105: 616-623, 1975
 3. R. A. Coulson, T. Hernandez and J. D. Herbert: Factors Affecting Amino Acid Transport In Vivo. International Symposium on Amino Acid Transport and Uric Acid, Innsbruck, Austria, June 1975 (publication pending)
 4. J. A. Antonioli and H. N. Christensen: A Mode of Intestinal Absorption Unusually Dependent on Physiological State. *Am. J. Physiol.* 215: 951-958, 1968
 5. D. H. Elwyn, H. C. Parikh and W. C. Shoemaker: Amino Acid Movements between Gut, Liver and Periphery in Unanesthetized Dogs. *Am. J. Physiol.* 215: 1260-1275, 1968
-

Bile Acid Excretion — A New Test of Functioning Liver Mass

Key Words: liver function test, cholic acid, bile salts

Bile salt metabolism has been actively studied in the past decade and seems clearly to be the substance excreted by the liver in the greatest number of moles per day. It seemed reasonable to look to a bile salt removal test as a measure of the total liver cell mass. The lack of a sensitive test for assay of serum bile salt levels and sources of pure bile salts had restricted this activity. However, a sensitive radioimmunoassay for serum bile acids and salts specific for conjugates of cholic acid¹ made such a test feasible.² Pure cholyglycine was prepared for the injection material. One to 7.5 μ Moles per kilogram of body weight were tested in normal subjects and all were cleared linearly in the first few minutes, but curvilinearly afterwards. A dose of 5 μ Moles per kilogram was eventually chosen for a consistent increase from fasting levels of cholyglycine ($0.44 \pm 0.8 \mu\text{M}$ to $4.35 \pm 0.5 \mu\text{M}$) one minute after injection and return to baseline after 12 minutes. Studies indicate that this material disappears at a rate of 2.6 minutes half life

as has been reported for indocyanine green and dibromosulfophthalein but much faster than bilirubin or sulfobromophthalein. This test validated in normals was applied to 36 patients with minimal to moderate degrees of liver abnormality. In 25 of these one or more conventional liver tests (including sulfobromophthalein retention at one hour) were abnormal and the fasting level of bile salt was elevated in all but five, and after 5 μ Moles per kilogram of body weight the levels at ten minutes of the half life were prolonged. In nine patients all liver tests were normal but the histological picture was abnormal. In all of these this test was abnormal. It is felt that this test offers a new and more sensitive method of testing liver function using a highly physiological parameter for the measurement. \square

1. W. F. Simmonds, M. G. Korman, V. L. W. Go and A. F. Hofmann: Radioimmunoassay of Conjugated Choly Bile Acid in Serum. *Gastroenterology* 65: 705-711, 1973
2. B. Josephson: The Circulation of Bile Acids in Connection with their Production, Conjugation and Excretion. *Physiol Rev.* 21: 463-468, 1941

Plasma Lipids and Lipoproteins in Vegetarians and Controls

Key Words: vegetarians, lipoproteins, cholesterol

A series of studies^{1,2} are emerging on a group of vegetarians who adhered to this diet for at least three years. The dietary staples in the 73 men and 43 women were whole grains, beans and fresh vegetables, with seaweed and fermented soy products consumed almost daily. Supplementary foods are fruits, nuts, beer and fish. Only

28 percent of the group consumed dairy products and 11 percent consumed eggs. No person used meat or poultry as frequently as weekly. The control group was chosen from the Framingham Heart Study Group using their children to obtain an age and sex matched group comparable to the vegetarians. These were estimated to eat a normal American diet. The mean cholesterol levels in milligrams per 100 ml was

126 vs 184, vegetarians vs normal, low density lipoprotein 73 vs 118, very low density lipoprotein 11.8 vs 17.2, high density lipoprotein 42 vs 49 and mean triglyceride levels 59 vs 86. The mean weight and subscapular skinfold thicknesses were 58 kg and 6 mm for vegetarians and 73 kg and 17 mm for controls. Similar differences in lipid levels were found between subgroups of 42 vegetarians and controls with identical mean weights. Multiple regression analyses showed that the consumption of dairy foods and eggs, but not body weight, was associated with the lipoprotein and cholesterol findings.

Although there are many possible explanations considered such as the caloric restriction and the use of macrobiotic foods, the authors propose that the vegetarian diet may in fact be more healthful for persons with hyperlipoproteinemia and hypertension than other therapeutic possibilities. □

1. F. M. Sacks, B. Rosner and E. H. Kass: Blood Pressure in Vegetarians. *Am. J. Epidemiol.* 100: 390-393, 1974
2. F. M. Sacks, W. P. Castelli, A. Donner and E. H. Kass: Plasma Lipids and Lipoproteins in Vegetarians and Controls. *New Engl. J. Med.* 292: 1148-1151, 1975

Severe Diarrhea in Children — New Ideas in an Unsolved Arena

Key Words: infantile diarrhea, *Escherichia coli*, rotaviruses, enterovirus, enterotoxins, childhood mortality

Diarrhea in the first two years of life appears to be a particularly severe illness because the colon has not yet fully developed its capacity to conserve water and electrolytes. In the past ten years it has become generally accepted that severe depleting infectious diarrheas result from stimulation of secretion and interference with absorption in the small intestine. A normal adult colon is a substantial protector of the organism from the severe depletions that occur in the young child. For reasons not very clear, but certainly widely written about, the malnourished and underprivileged seem to be far more exposed to these problems than others.

Enterotoxigenic *Escherichia coli* has been identified as a cause of severe human diarrhea for only a few years^{1,3} and almost no studies exist of the true prevalence. Certain strains of *E. coli* are postulated to produce a cholera-like enterotoxin and this is the basis of the diarrhea. Gorbach and his associates⁴ using the infant rabbit model to demonstrate enterotoxins of both the heat labile and heat stable form found 83 percent of Chicago infants harbored strains

that gave positive responses. Sack and his co-workers⁵ exhaustively studied the *E. coli* problem in 64 episodes of acute diarrhea in 59 Apache children hospitalized for diarrhea. Each child had isolates of intestinal bacteria and strains of *E. coli*. Nineteen children had cultures of the small bowel in addition to stools. Isolates of *E. coli* were verified and serotyped. At least five and up to ten separate isolation colonies of *E. coli* were pooled for subsequent studies. Three separate sensitive assays of enterotoxin production were utilized. These included the 18 hour rabbit ileal loop assay, the baby rabbit test assay and the adrenal cell assay utilizing mouse adrenal tissue culture cells. A total of 934 *E. coli* isolates were obtained. An enterotoxin was isolated in ten of the 64 diarrhea episodes. These patients had no clinical characteristics that differentiated them from other causes of diarrhea. The adult loop test and the adrenal cell test were excellent screening tests, but there were four false/negative results in the infant rabbit assay. None of the 35 enterotoxigenic strains assayed were invasive by the Sereny test. Serotyping indicated that the types responsible for disease were highly varied (eight different types were found)

and these were not included in the lists usually put forth of enteropathogenic *E. coli*.

It is concluded that this accounted for 16 percent of the diarrhea but that a specific assay for enterotoxin is essential. This suggests strongly that these complex tests must be undertaken in any study of infantile diarrhea.

A second avenue of interest in infantile diarrhea has come from studies directly visualizing fecal viruses. Viral enteritis in calves, an important domestic cattle disease, revealed massive numbers of viral particles in the stool of these animals.⁶ Applying similar techniques Bishop and co-workers found in the duodenal tissue of infants with severe gastroenteritis reovirus-like particles in the mucosal cells and later showed these in the stool.^{7,8} Many groups are now working on this problem. Techniques to show specificity include reaction of the stool with acute and convalescent phase sera which produces agglutination of the virus in stool. It is now apparent that these techniques are successful in identifying rotaviruses, enteroviruses and adenoviruses.⁹ It is apparent that investigation of diarrhea in infants is changing in its methodology and that rotavirus infection and enterotoxigenic *E. coli* are two important diseases that may have been previously overlooked. □

1. S. L. Gorbach, J. G. Banwell, B. D. Chatterjee, B. Jacobs and R. B. Sack: Acute Undifferent-

iated Human Diarrhea in the Tropics. I. Alterations in Intestinal Micro-Flora. *J. Clin. Invest.* 50: 881-889, 1971

2. R. B. Sack, S. L. Gorbach, J. G. Banwell, B. Jacobs, B. D. Chatterjee and R. C. Mitra: Enterotoxigenic *Escherichia coli* Isolated from Patients with Severe Cholera-Like Disease. *J. Infect. Dis.* 123: 378-385, 1971
3. H. L. Dupont, S. B. Formal, R. B. Hornick, M. J. Snyder, J. P. Libonati, D. G. Sheahan, E. H. LaBrec and J. P. Kalas: Pathogenesis of *Escherichia coli* Diarrhea. *New Eng. J. Med.* 285: 1-9, 1971
4. S. L. Gorbach and C. M. Khurana: Toxigenic *Escherichia coli*. A Cause of Infantile Diarrhea in Chicago. *New Engl. J. Med.* 287: 791-795, 1972
5. J. D. Almeida: Visualization of Fecal Viruses. *New Engl. J. Med.* 292: 1403-1404, 1975
6. A. L. Fernelius, A. E. Ritchie and L. G. Classick: Cell Culture Adaptation and Propagation of a Reovirus-Like Agent of Calf Diarrhea from a Field Outbreak in Nebraska. *Arch. Gesamte Virusforsch.* 37: 114-130, 1972
7. R. F. Bishop, G. P. Davidson, I. H. Holmes and B. J. Ruck: Virus Particles in Epithelial Cells of Duodenal Mucosa from Children with Acute Non-Bacterial Gastroenteritis. *Lancet* II: 1281-1283, 1973
8. R. F. Bishop, G. P. Davidson, I. H. Holmes and B. J. Ruck: Detection of a New Virus by Electron Microscopy of Faecal Extracts from Children with Acute Gastroenteritis. *Lancet* I: 149-151, 1974
9. T. H. Flewett, A. S. Bryden, H. Davies and C. A. Morris: Epidemic Viral Enteritis in a Long-Stay Children's Ward. *Lancet* I: 4-5, 1975

Neuropathy in Renal Patients on Chronic Dialysis

Key Words: uremia, polyneuritis, deafness, renal transplant

Approximately half of the patients with renal failure on chronic dialysis have peripheral neuropathy of some degree.¹ Although most of this disease is asymptomatic and therefore only important if it worsens, it has been apparent that uremic patients with severe paralytic forms of peripheral neuropathy make clinically satisfactory recovery when they receive

transplants. The cause of the neuropathy is really unknown but there has been speculation that there are specific toxins, which are not effectively dialyzed and therefore a search for a larger pore membrane has been made with little or no success.^{2,3}

An important study of uremic deafness has appeared⁴ in which serial study of hearing loss was made using an audiometer. They found that the uremic inner ear deafness was symmetrical and of the coch-

leobasal type. The short increment sensitivity index was positive in all cases (80 to 100 percent). Mild deterioration was observed in the patients while undergoing dialysis but six to 26 months following the successful renal transplantation the patients showed improvement. The hearing loss in the low range averaged 18 to 25 decibels; in the higher range it was 25 to 40 decibels and it decreased to 15 or less. The short increment sensitivity index returned to within 10 percent of normal. The neuropathy of uremia poses an increasing problem that requires more work. At the moment the toxin does not seem to be a known nutritional agent. □

1. P. J. Dyck, W. J. Johnson, E. H. Lambert, W. Bushek and M. Pollock: Detection and Evaluation of Uremic Peripheral Neuropathy in Patients on Hemodialysis. *Kidney Int.* 7: S201-S205, 1975
2. J-L. Funck-Brentano, N. K. Man and A. Sausse: Effect of More Porous Dialysis Membranes on Neuropathic Toxins. *Kidney Int.* 7: S52-S57, 1975
3. C. M. Kjellstrand, R. J. Petersen, R. L. Evans, J. R. Shideman, B. von Hartitzsch and T. J. Buselmeir: Considerations of the Middle Hypothesis II: Neuropathy in Nephrectomized Patients. *Trans. Am. Soc. Art. Int. Org.* 19: 325-336, 1973
4. H. Mitschke, P. Schmidt, H. Kopsa and J. Zazgornik: Reversible Uremic Deafness after Successful Renal Transplantation. *New Engl. J. Med.* 292: 1062-1063, 1975

Fellowships in Clinical Nutrition

The Department of Nutrition and Food Science, Massachusetts Institute of Technology and the Children's Hospital Medical Center, Boston, Massachusetts, will offer fellowships in clinical nutrition starting July, 1976. The program is multidisciplinary in nature, providing physicians with an opportunity to acquire a broad background in clinical nutrition. The program's principal objective is the training of independent investigators for research in clinical nutrition and teaching of applied nutrition to house staff and medical students. The training program includes courses and seminars in nutrition at M.I.T. as well as clinical activities at Children's Hospital, the New England Deaconess Hospital and Boston University Hospital. The fellowship will be offered for two or three years depending on the interests of the individual. Two or three years of post M.D. specialty training in pediatrics, internal medicine or surgery is required. Applicants must be citizens or non-citizen nationals of the United States, or have been lawfully admitted to the United States for permanent residence and have in their possession a permanent visa at time of application. Send all requests for information to Dr. Robert M. Suskind, Program Director, Clinical Nutrition Fellowship, Department of Nutrition and Food Science, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139. □

Announcement of Meetings

The American Diabetes Association announced its 1976 schedule of professional meetings.

The 23rd Postgraduate Course, "Diabetes in Review: Clinical Conference 1976" will be held January 28-30, 1976, at the Sheraton Inn, Boston, Massachusetts.

The 36th Annual Meeting of the American Diabetes Association will be held June 20-22, 1976 at the Hilton Hotel, San Francisco, California.

The Eighth Allied Health Postgraduate Course in Diabetes will be held October 4-6, at the Sheraton Hotel, Philadelphia, Pennsylvania.

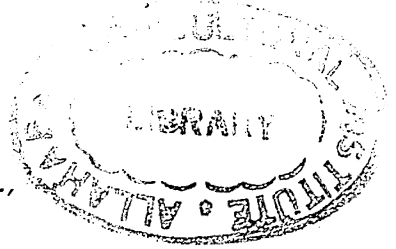
For further information regarding any of these meetings contact:

Ernest M. Frost, Ed.D.
Executive Vice-President
1 West 48th Street
New York, New York 10020

6 JAN 1976

Niacin

by William J. Darby, M.D., Ph.D.,
Kristen W. McNutt, Ph.D. and
E. Neige Todhunter, Ph.D.



In 1867 a compound, later named¹ nicotinic acid, was obtained² by oxidation of nicotine. It was found per se in nature in 1912 in an anti-beriberi concentrate of rice polishings,³ and repeatedly associated with vitamin-like effects by Casimir Funk and others⁴⁻⁷ during the period from 1913 to 1937. In 1937 it was identified with the pellagra-preventive (anti-canine blacktongue) factor.⁸⁻¹² Nicotinamide had been demonstrated to be a moiety of coenzymes I¹³ and II¹⁴ (di- and tri-phosphopyridine nucleotide, respectively) and nicotinic acid found to be a growth factor for streptococcus.¹⁵ The two nicotinamide-containing coenzymes are involved in glycolysis, fat synthesis and tissue respiration.

Nomenclature

Niacin is accepted as "the generic description for pyridine 3-carboxylic acid and derivatives exhibiting qualitatively the biological (nutritional) activity of nicotinic acid. Thus phrases such as 'niacin activity' and 'niacin deficiency' represent preferred usage. The compound pyridine 3-carboxylic acid . . . , also known as niacin or

vitamin PP, should be designated nicotinic acid."^{16,17} Nicotinamide is the amide of nicotinic acid, or "niacinamide." "Nicotinamide equivalent" is the contribution to the dietary intake of all different nutritionally active forms of niacin (including tryptophan conversion to niacin) and is expressed in terms of milligram (or microgram) equivalents of nicotinamide.¹⁶⁻¹⁸

Pellagra

Pellagra, now known to result from niacin deficiency, appeared in Europe¹⁹ when corn (*zea mays*) from the New World became the major staple foodstuff of successive regions around the Mediterranean—in Spain, France, Italy and eastward. Described by Gaspar Casal as endemic from the 1730's in the Austurian region of Oviedo, it acquired many descriptive designations,²⁰ including "mal de la Rosa," "mal del Sol" (illness of the sun), "vernal insolation" (sunburn of the spring), "corn bread fever," and "pellagra" (pě-lă'-gră, or pě-lăg'-ră—*pelle*, skin, and *agra*, rough). It was recognized in the U.S. about the turn of this century and caused hundreds of thousands of cases of morbidity and death in the Midwest and South—from Illinois to South Carolina. Victims suffered "the 3-D's"—dermatitis of areas exposed to the sun, diarrhea, and dementia. Several mental institutions in the U.S., Europe and Egypt were primarily devoted to care of pellagrins.

In 1914 Joseph Goldberger, a bacteriologist with the U.S. Public Health Service, was assigned the task of identifying the cause of the disease. In classic epidemio-

Dr. Darby is President of The Nutrition Foundation. Dr. McNutt is Research Associate with The Nutrition Foundation. Dr. Todhunter is Visiting Professor of Nutrition at the Vanderbilt University School of Medicine, Nashville, Tennessee 27203.

A limited number of reprints of this article may be obtained from The Nutrition Foundation, 888 Seventeenth Street, N.W., Washington, D.C. 20006. THERE ARE NO REPRINTS OF UNSIGNED REVIEWS.

logic studies,²¹ he noted the association of the disease with poor diet—"the 3-M's"—meal (corn), meat (fat back) and molasses—plus poverty. He observed that well-fed persons did not contract the disease. He reproduced the condition in convicts in Mississippi by feeding them a pellagrogenic diet. He, his wife and 14 volunteer colleagues constituted a "filth squad" who ingested and were injected with various biological materials and/or excreta from pellagrins, thus demonstrating the non-infectious nature of pellagra. In orphanages, prisons and mental institutions the therapeutic value of a good diet was demonstrated. Foods were assayed in man for their pellagra-curative properties, and for their pellagra-preventive activity in the animal model of canine blacktongue.

Tryptophan as Niacin Precursor

Elucidation of the metabolism of tryptophan, its conversion to niacin metabolites, and the classic studies of Grace Goldsmith and associates²²⁻²⁷ and of Horwitt and associates²⁸ in man established the basis of a quantitative estimate of the nutritional interrelationship. Evidence from these human studies is widely interpreted to indicate that in man 60 mg of tryptophan has an average niacin equivalency of 1 mg of nicotinamide. Accordingly, estimated human requirements of niacin must take into account the tryptophan content of the diet as well as nicotinic acid and nicotinamide. This ratio may not apply in absence of dietary niacin or with limited dietary tryptophan.²⁹ Vivian et al^{30,31} found that urinary excre-

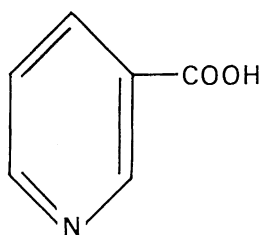


Figure 1. Nicotinic acid

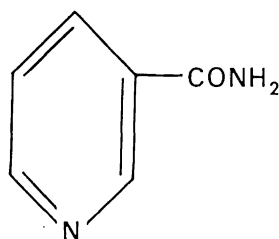


Figure 2. Nicotinamide

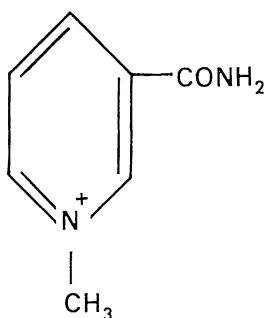


Figure 3. N¹-Methylnicotinamide

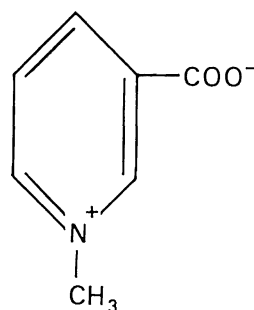


Figure 4. Trigonelline (N¹-methylnicotinic acid betaine)

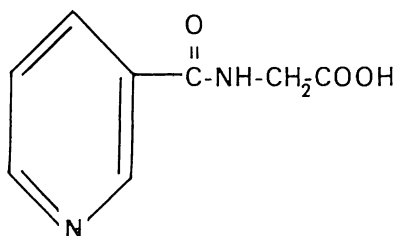


Figure 5. Nicotinuric acid (nicotinyglycine)

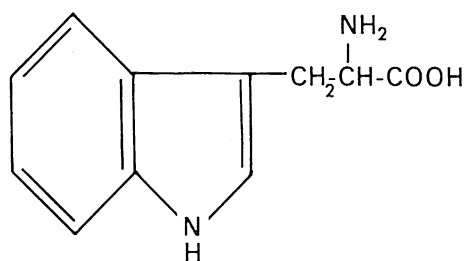


Figure 6. L-Tryptophan

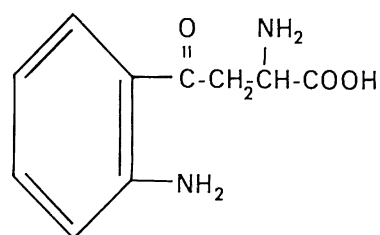


Figure 7. L-Kynurenine

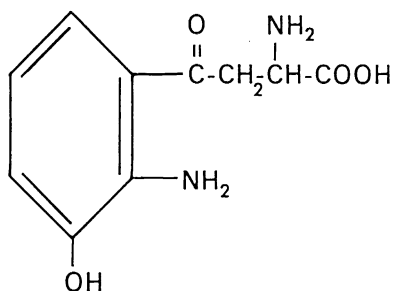


Figure 8. L-3-Hydroxykynurenine

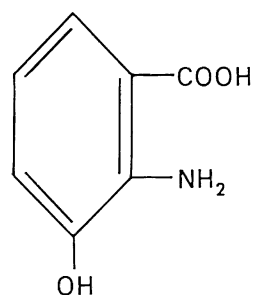


Figure 9. 3-Hydroxyanthranilic acid

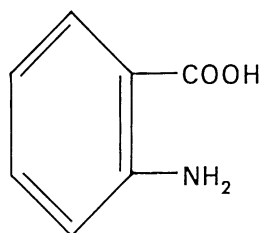


Figure 10. Anthranilic acid

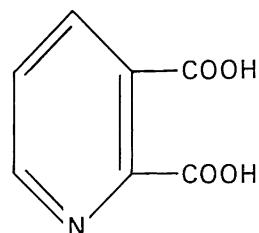


Figure 11. Quinolinic acid

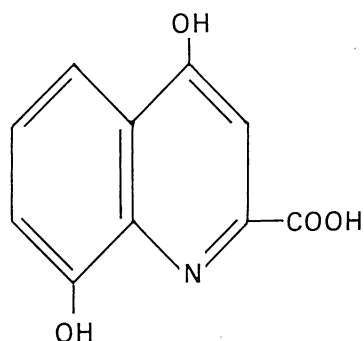


Figure 12. Xanthurenic acid

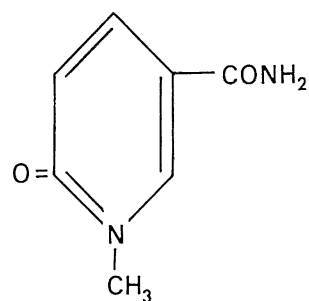


Figure 13. N¹-methyl-2-pyridone-5-carboxylamide (2-pyridone)

tion of metabolites of niacin or those of the tryptophan-niacin pathway did not fall to zero even when tryptophan intake was very low. Increasing amounts of dietary tryptophan are used first to restore nitrogen balance, next to restore blood pyridine nucleotides, and then to be excreted as niacin metabolites.

The understanding of the quantitative relationships for conversion of tryptophan to niacin during pregnancy is particularly unsatisfactory. Data³² on urinary excretion of metabolites have been interpreted to indicate that the conversion of this amino acid to niacin is more efficient during pregnancy, and hence, that the niacin equivalency of tryptophan is greater in the gravid woman. This may be misinterpretation of observations as it long has been known that early in pregnancy there is a pronounced increment in the urinary excretion of N-methylnicotinamide.³³ Another interpretation may be that more tryptophan is diverted via this route or that methylation of niacin is enhanced during pregnancy and therefore that the dietary requirement is in fact increased.

The structures for 13 known metabolites of niacin are presented on pages 290 and 291.

Bound Forms of Niacin

Additional evidence now indicates the presence in many foodstuffs, especially cereals, of niacin-containing compounds from which the niacin is not nutritionally available.³⁴⁻³⁸ Initial evidence stemmed from the observed discrepancy between values for niacin content in cereals as determined colorimetrically before and after hydrolysis with dilute NaOH. Microbiological assays similarly indicated that some 20 percent more niacin was obtained in dilute alkaline extracts of cereals than in aqueous or acid extracts. These increases were consistent with the interpretation of "bound forms" in the cereals. Later studies showed that the bound niacin was ineffective in curing niacin deficiency in the rat, chick, duck or pig. Evidence for man is not so clear, but a discrepancy between the total niacin content of the high corn diet

used by Goldberger to produce experimental pellagra and the lower intake of healthy population groups in North Carolina was early noted by W. J. Dann.³⁹

Two types of bound niacin³⁴⁻³⁸ were initially described: (1) a peptide with molecular weight of 12,000 to 13,000, so-called niacinogens; and (2) a carbohydrate complex with a molecular weight of some 2,370. The name niacytin has been used to designate this latter material from wheat bran. It now appears that there may be a number of niacin-containing bound forms in wheat⁴⁰ and that niacytin preparations contain peptides, hexoses and pentoses. Resolution of the important problems of the relative activities of precursors and the biological availability of forms of niacin in foods deserves high priority.

Leucine Effects

An additional nutritional concept relative to niacin requirements pertains to the reported effect of high intake of leucine in precipitating pellagra, initially described as a result of a millet (jowar) based diet. Jowar (*sorghum vulgare*), which contains much presumably available niacin, is not low in tryptophan but is high in leucine. Belavady and Gopalan⁴¹ noted that jowar, fed as 65 percent of a diet, produced black-tongue in dogs. The same laboratory has reported⁴² that the addition of leucine to a casein-based ration enhanced urinary excretion of quinolinic acid (a tryptophan metabolite) in rats, and that the addition of 1 mg of niacin per 100 g of ration partially reduced this increase and the addition of 100 mg of tryptophan did not alter it. In man^{43,44} they reported a similar increase of urinary quinolinic acid and an accompanying decrease in excretion of N-methylnicotinamide and 6-pyridone after administration of leucine to normal subjects. They further reported that a 10 g daily supplement of L-leucine decreased the ability of erythrocytes of normal subjects and pellagrins to synthesize nicotinamide nucleotides when incubated with niacin. However, there were no differences in erythrocyte nucleotides of pellagrins and non-pellagrins.

Dietary Allowances

Despite these uncertainties, the current estimates^{4,5-4,8} of adult human needs for niacin likely are sound guidelines because they are based upon intakes of subjects who, under either conditions leading to endemic pellagra or experimentally induced disease, manifested evidences of niacin deficiency, along with accompanying biochemical and dietary analyses, and because they include reasonable allowances for niacin equivalents. The Recommended Dietary Allowances (RDA, 1974)^{4,5} for adults, expressed as niacin, is 6.6 mg per 1000 kcal, and not less than 13 mg at caloric intakes of less than 2000 kcal. The FAO/WHO guidelines for nutritional requirements (1974)^{4,6,4,8} are essentially the same. The RDA^{4,5} notes that:

There are no data on the niacin requirements of children from infancy through adolescence. The possibility exists that the efficiency of tryptophan conversion to niacin in rapidly growing children may differ from that observed in adults... Human milk contains approximately 0.17 mg of niacin and 22 mg of tryptophan per 100 ml or 70 cal.^{4,9} The niacin allowance recommended for infants up to six months is 8 mg per 1000 kcal, about two-thirds of which will ordinarily come from tryptophan; and for children over six months and adolescents, 6.6 mg per 1000 kcal, but not less than 8 mg daily.

There is no information on the niacin requirements of pregnant and lactating women... The allowance recommended provides an increase of 2 mg of niacin daily during pregnancy, based on the recommended increase in energy intake. For lactation, an additional daily allowance of 4 mg of niacin is recommended, consistent with the additional allowance of 500 kcal.

These are summarized in Table 1. Allowances for children and pregnant women are based upon less direct evidence, but again in practice carry the convincing weight of observed protectiveness against recognizable deficits.

Hartnup's Disease

A rarely occurring familial (homozygous) deviation of niacin-tryptophan metabolism that produces a clinical syn-

Table 1
Recommended Dietary Allowances of Niacin

	Age in years	RDA (mg)
Infants	0.0-0.5	5
	0.5-1.0	8
Children	1-3	9
	4-6	12
	7-10	16
Males	11-14	18
	15-18	20
	19-22	20
	23-50	18
	51+	16
Females	11-14	16
	15-18	14
	19-22	14
	23-50	13
	51+	12
Pregnant		+2
Lactating		+4

drome mimicking pellagra is known as Hartnup's disease (originally H disease),^{5,0} characterized by a photosensitivity and dermatitis, ataxia and psychiatric changes, and massive aminoaciduria involving monoamino-monocarboxylic acids.^{5,1} Patients have diminished intestinal absorption of these amino acids, as well as decreased tubular resorption of them, with resulting increased intestinal decomposition of tryptophan. Oral ingestion of the latter leads to urinary excretion of large quantities of indoxyl sulfate (indican), indolylacetic acid, and indolylacryloglycine, all products of intestinal bacterial action on tryptophan. The patients have impaired ability to convert tryptophan to kynurenine and nicotinamide, due at least in part to the absorptive defect. In general they respond satisfactorily to administered niacin (nicotinamide). Study of this condition, described in 1952 and named for the first family in which it was recognized, further solidifies the importance of the concept of tryptophan as a precursor of niacin in man.

Pharmacologic Effects

Massive doses of nicotinic acid (but not the amide) early were observed to produce vascular dilatation or "flushing," with accompanying sensation of burning or stinging of the face and hands. This pharmacologic effect varies from person to person and is said to be reduced by simultaneous ingestion of glycine. It has been used therapeutically in efforts to induce cerebrovascular dilatation in senile ataxia, and as a "harmless" placebo in management of some hypochondriacs. Dosing with nicotinic acid of infants with kwashiorkor (mistakenly called "infantile pellagra") proved ineffective or injurious,^{5,2} probably because of the demand created by the added niacin for increased methylation in a state of protein deficiency with, often, pronounced hepatic injury.

An extensively studied pharmacologic property of niacin is the lowering of serum cholesterol and lipoprotein concentrates by massive (usually 3 or more grams per day) oral doses of nicotinic acid.^{5,3,5,4} Nicotinamide does not share this effect. Reduced levels of free and esterified cholesterol and of β -lipoprotein cholesterol fraction occurs. Substitution of nicotinamide does not maintain the effect. Among the metabolic effects of large doses of nicotinic acid two seem noteworthy: the decreased mobilization of fatty acids from adipose tissue in exercising subjects^{5,5} and the increased utilization of muscle glycogen stores.^{5,6} Similar effects on cardiac muscle metabolism have now been reported, i.e., inhibition of use of free fatty acids as energy substrates by human myocardial tissue and depletion of glycogen and muscle fat through increased myocardial utilization.^{5,7} Such evidence together with earlier observations^{5,3,5,4} suggesting that long-term high-dose nicotinic acid therapy may in some instances be associated with appearance of laboratory evidence of diabetes and of hepatic injury, as well as activation of peptic ulcers (with use of the unbuffered acid) all emphasize the non-physiologic levels of niacin involved.

The Coronary Drug Project Research Group^{5,8} studied the efficacy and safety of several lipid-influencing drugs in long-term therapy of coronary heart disease in men with proven previous myocardial infarctions. Fifty-three project clinical centers recruited 8,341 patients and compared in a double-blind manner the therapeutic agents with a lactose placebo. Niacin in doses of 3.0 g per day was received by 1,119 subjects. The treatment periods ranged from beginning of therapy between March 1966 and October 1969 to termination during June through August 1974, with a mean therapeutic time of 74 months. Conclusions of the project relative to niacin were:^{5,8}

... there is no evidence of efficacy of niacin with regard to total mortality. The five-year total mortality for niacin was slightly higher than that for the placebo group (21.2% vs 20.9%). For the total follow-up experience, the niacin group had a somewhat—but not statistically significant—lower mortality (24.4%) than the placebo group (25.4%). No sub-group of patients was identified in which niacin showed a beneficial effect with respect to five-year total mortality. The niacin group did experience a statistically significant lower incidence of definite, nonfatal myocardial infarction than the placebo group; the five-year rates were 8.9% for niacin and 12.2% for placebo, while the incidence for the total follow-up experience was 10.2% for niacin and 13.8% for placebo. . . . Data from this study confirmed previously reported findings of increased incidence of gastrointestinal problems and elevated levels of serum enzymes, serum uric acid, and plasma glucose in men taking niacin. The long-term clinical significance of these chemical changes is unknown.

In conclusion, the Coronary Drug Project data yield no evidence that niacin influences mortality of survivors of myocardial infarction; this medication may be slightly beneficial in protecting persons to some degree against recurrent nonfatal myocardial infarction. However, because of the excess incidence of arrhythmias, gastrointestinal problems, and abnormal chemistry findings

in the niacin group, great care and caution must be exercised if this drug is to be used for treatment of persons with coronary heart disease.

Megavitamin Therapy

Over the past 20 years, some therapists have advocated so-called megavitamin therapy for certain psychiatric ills.^{5,9} This originated with a mode of treatment proposed for schizophrenia employing massive doses of niacin (termed by the advocates vitamin B₃). Later, advocates of megavitamin therapy have added a variety of other derivatives and vitamins in massive amounts to their proposed armamentarium, as well as a widening variety of diseases of obscure origin which they propose for treatment or prevention. The therapeutic regimen or concept has now been termed "orthomolecular treatment."

The Task Force on Vitamin Therapy in Psychiatry of the American Psychiatric Association has examined the evidence relative to this matter and concludes^{5,9} that:

... the results and claims of the advocates of megavitamin therapy have not been confirmed by several groups of psychiatrists and psychologists experienced in psychopharmacological research... The theoretical basis for megavitamin treatment especially with nicotinic acid has been examined and found wanting... under these circumstances this Task Force considers the massive publicity which they promulgate via radio, the lay press and popular books, using catch phrases which are really misnomers like 'megavitamin therapy' and 'orthomolecular treatment' to be deplorable.

Biochemical Assessment of Nutriture

The metabolic basis for the clinical biochemical assessment of niacin nutriture is the excretion of two metabolites of niacin:^{6,0} N¹-methylnicotinamide and N¹-methyl-2-pyridone-5-carboxylamide (2-pyridone). Normally adults excrete 20 to 30 percent of their niacin as the N-methyl and 40 to 60 percent as the 2-pyridone metabolites. Sauberlich et al^{6,0} consider the most promising procedure for evaluation of nutriture to be use of the 2-pyridone/N¹-methylnicotinamide urinary excretion ratio since the ratio of

1.3-4.0 usually exists but as depletion occurs the 2-pyridone is absent for weeks before clinical signs are noted while the N¹-methylnicotinamide excretion falls to a minimum at about the time clinical signs are evident. Hence, a ratio value of less than 1.0 is suggested by DuPlessis^{6,1} and de-Lange and Joubert^{6,2} as indicative of latent niacin deficiency. For survey studies measurement of N¹-methylnicotinamide has been most widely employed and is convenient. However, the method suffers from poor reproducibility in the hands of some workers and from the frequent presence in urine of interfering compounds. Imperfect though they are, these procedures do permit the monitoring of successive stages of depletion of subjects and the assessment of dietary intakes of population groups in well designed nutrition surveys.^{6,3} The analysis for 2-pyridone, however, is tedious and time consuming. Estimations of blood serum or cellular levels of nicotinamide nucleotides have not proved to be fruitful methods of assessing nutriture. The further refinement of biochemical assessment of niacin nutriture will likely result from improved analytical methodology and better information relative to the variations of the metabolites during differing levels of nutriture. □

1. H. Weidel, *Annal. der Chemie u. Pharmacie* 165: 328, 1873
2. C. Huber, *Annal. der Chemie u. Pharmacie* 141: 271, 1967
3. U. Suzuki, T. Shimamura and S. Otake, *Biochem. Zeitschr.* 43: 89-153, 1912
4. C. Funk, *J. Physiol.* 46: 173-179, 1913
5. C. Funk and I. C. Funk, *J. Biol. Chem.* 119: xxxv-xxxvi, 1937
6. J. C. Drummond and C. Funk, *Biochem. J.* 8: 598-615, 1914
7. H. B. Vickery, *J. Biol. Chem.* 68: 585-592, 1926
8. C. A. Elvehjem, R. J. Madden, F. M. Strong and D. W. Woolley, *J. Am. Chem. Soc.* 59: 1767-2768, 1937
9. P. J. Fouts, O. M. Helmer, S. Lepkovsky and T. H. Jukes, *Proc. Soc. Exp. Biol. Med.* 37: 405-407, 1937

10. D. T. Smith, J. M. Ruffin and S. G. Smith, *J. Am. Med. Assn.* 109: 2054-2055, 1937
11. L. J. Harris and A. Hassan, *Lancet* II: 1467, 1937
12. D. W. Woolley, F. M. Strong, R. J. Madden and C. A. Elvehjem, *J. Biol. Chem.* 124: 715-723, 1938
13. H. von Euler, H. Alhers and F. Schlenk, *Hoppe-Seyler's J. Physiol. Chem.* 240: 113-126, 1936
14. O. Warburg and W. Christian, *Biochem. Zeitschr.* 274: 112-116, 1934; 275: 112, 464, 1935
15. B. C. J. G. Knight, *Biochem. J.* 31: 731-737, 1937
16. AIN Committee on Nomenclature, *J. Nutrition* 105: 134-140, 1975
17. E. N. Todhunter: *A Guide to Nutrition Terminology for Indexing and Retrieval*. Pp. 270. U. S. Government Printing Office, Washington, D. C., 1970
18. IUNS Committee on Nomenclature, *Nutrition Abst. Rev.* 40: 395-400, 1970
19. A. Hirsch: *Handbook of Geographical and Historical Pathology*. Translated from the second German Edition by C. Creighton. Pp. 217-253, vol. II. The New Sydenham Society, London, 1885
20. G. M. Niles: *Pellagra, An American Problem*. Pp. 253. W. B. Saunders Co., Philadelphia and London, 1912
21. M. Terris: *Goldberger on Pellagra*. Pp. 395. Louisiana State University Press, Baton Rouge, 1964
22. H. P. Sarett and G. A. Goldsmith, *J. Biol. Chem.* 167: 293-294, 1947
23. G. A. Goldsmith, H. P. Sarett, U. D. Register and J. Gibbens, *J. Clin. Invest.* 31: 533-542, 1952
24. G. A. Goldsmith, H. L. Rosenthal, J. Gibbens and W. G. Unglaub, *J. Nutrition* 56: 371-386, 1955
25. G. A. Goldsmith, J. Gibbens, W. G. Unglaub and O. N. Miller, *Am. J. Clin. Nutrition* 4: 151-160, 1956
26. G. A. Goldsmith, *J. Am. Dietet. Assn.* 32: 312-316, 1956
27. E. Z. Moyer, G. A. Goldsmith, O. N. Miller and J. Miller, *J. Nutrition* 79: 423-430, 1963
28. M. K. Horwitt, C. C. Harvey, W. S. Rothwell, J. L. Cutler and D. Haffron, *J. Nutrition* 60 (Suppl. 1) 1-43, 1956
29. *Nutrition Reviews* 32: 76-77, 1974
30. V. M. Vivian, M. M. Chaloupka and M. S. Reynolds, *J. Nutrition* 66: 587-598, 1958
31. R. R. Brown, V. M. Vivian, M. S. Reynolds and J. M. Price, *J. Nutrition* 66: 599-606, 1958
32. A. E. Wertz, M. E. Lojkin, B. S. Bouchard and M. B. Derby, *J. Nutrition* 64: 339-353, 1958
33. W. J. Darby, W. J. McGanity, M. P. Martin, E. Bridgforth, P. M. Densen, M. M. Kaser, P. J. Ogle, J. A. Newbill, A. Stockell, M. E. Ferguson, O. Touster, G. S. McClellan, C. Williams and R. O. Cannon, *J. Nutrition* 51: 565-597, 1953
34. *Nutrition Reviews* 15: 11-12, 1957
35. *Nutrition Reviews* 19: 240-242, 1961
36. *Nutrition Reviews* 23: 286-287, 1965
37. *Nutrition Reviews* 29: 260-262, 1971
38. *Nutrition Reviews* 32: 124-125, 1974
39. W. J. Dann, *Fed. Proc.* 3: 159-161, 1944
40. J. B. Mason, N. Gibson and E. Kodicek, *Brit. J. Nutrition* 30: 297-311, 1973
41. B. Belavady and C. Gopalan, *Lancet* II: 1220-1221, 1965
42. N. Raghuramulu, B. S. Narasinga Rao and C. Gopalan, *J. Nutrition* 86: 100-106, 1965
43. N. Raghuramulu, S. G. Srikantia, B. S. Narasinga Rao and C. Gopalan, *Biochem. J.* 96: 837-839, 1965
44. B. Belavady, S. G. Srikantia and C. Gopalan, *Biochem. J.* 87: 652-655, 1963
45. *Recommended Dietary Allowances*. Eighth edition. Food and Nutrition Board, National Academy of Sciences, Washington, D. C., 1974
46. R. Passmore, B. M. Nicol and M. N. Rao in collaboration with G. H. Beaton and E. M. DeMayer: *Handbook on Human Nutritional Requirements*. FAO and WHO, Food and Agriculture Organization of the United Nations, Rome, 1974
47. *Recommended Daily Nutrient Intakes*. Health and Welfare Canada. Committee for Revision of the Canadian Dietary Standard, Bureau of Nutritional Sciences, revised 1974
48. *Nutrition Reviews* 33: 147-157, 1975
49. K. U. Toverud, G. Stearns and I. G. Macy: *Maternal Nutrition and Child Health*. NRC Bulletin 123, pp. 174, National Academy of Sciences, Washington, D. C., 1950
50. D. N. Baron, C. E. Dent, H. Harris, E. W. Hart and J. B. Jepson, *Lancet* II: 421-428, 1956
51. J. B. Jepson in *The Metabolic Basis of Inherited Disease*. J. B. Stanbury, J. B. Wyngaarden and D. S. Fredrickson, Editors, pp. 1486-1503, third edition. McGraw-Hill, New York, 1972

52. H. C. Trowell, J. N. P. Davies and R. F. A. Dean: *Kwashiorkor*. Pp. 308. Edward Arnold, London, 1954
53. *Nutrition Reviews* 17: 168-169, 1959
54. *Nutrition Reviews* 19: 325-328, 1961
55. L. A. Carlson, R. J. Havel, L. G. Ekelund and A. Holmgren, *Metabolism* 12: 837-845, 1963
56. J. Bergstrom, E. Hultman, L. Jorfeldt, B. Pernow and J. Wahren, *J. Appl. Physiol.* 26: 170-176, 1969
57. *Nutrition Reviews* 31: 80-81, 1973
58. The Coronary Drug Project Research Group, *J. Am. Med. Assn.* 231: 360-381, 1975
59. *Megavitamin and Orthomolecular Therapy in Psychiatry*. American Psychiatric Association Task Force on Vitamin Therapy in Psychiatry. Publications Services Division, American Psychiatric Association, Washington, D.C., 1973
60. H. E. Sauberlich, J. H. Skala and R. P. Dowdy: *Laboratory Tests for the Assessment of Nutritional Status*. CRC Press, Inc., Cleveland, 1974
61. J. P. DuPlessis: *An Evaluation of Biochemical Criteria for Use in National Nutrition Surveys*. Council for Scientific and Industrial Research, Report No. 261, National Nutrition Research Institute, Pretoria, South Africa, 1967.
62. D. J. de Lange and C. P. Joubert, *Am. J. Clin. Nutrition* 15: 169-174, 1964
63. *Manual for Nutrition Surveys*. Second edition, Interdepartmental Committee on Nutrition for National Defense, U.S. Government Printing Office, Washington, D.C., 1963.

Nutrition Reviews

authoritative articles by distinguished international scientists • competent, critical, and knowledgeable reviews • interpretative summaries of important original clinical and experimental research • selected excerpts from classic publications marking milestones in nutrition research • informative special reports on government and organization actions • contributing editors review journals of widespread interest covering biochemistry, food science, public health, toxicology, dentistry, pediatrics, internal medicine, gastroenterology and other clinical specialties •

Send subscription and renewal requests to:

THE NUTRITION FOUNDATION
888 Seventeenth Street, N.W.
Washington, D. C. 20006

\$12.00 per year

\$6.00 for students

VITAMIN LEVELS AT TERM AND IN THE NEONATE

Nine water-soluble vitamins were injected into mothers shortly before delivery. Subsequent levels in their babies were then compared with the maternal levels.

Key Words: vitamins, neonatal, maternal, term

The fetus inevitably takes its mother for better or for worse with regard to nutrition. A previous study¹ showed that levels of water-soluble vitamins, although higher in the newborn infant, are related to the levels in maternal circulation at delivery.

The present study by Kaminetzky and his co-workers² is an amplification of this previous work. Forty-six women in labor were given an intravenous injection of vitamins after blood had been withdrawn for baseline studies. The vitamins given were thiamin, riboflavin, niacinamide, pyridoxine, pantothenate, biotin, vitamin C, folic acid and cyanocobalamin. Immediately after delivery, samples of maternal and cord blood were obtained and levels of each vitamin measured. The baseline levels were compared with blood vitamin levels determined in two previous studies; first, of random hospital patients where levels constituting 'hypovitaminemia' were defined³ and second, of well-nourished pregnant women who were found to have values within the non-pregnant range.⁴ It was necessary to include data from the previous paper³ since all the mothers were given vitamin injections. Thus cord blood levels at time zero, i.e. before injection, had to be derived from the previous study. Maternal and cord blood levels of each vitamin were followed for up to five hours. The number of observations at one, two and five hours depended on the interval between the injection of the vitamins and delivery.

Within this study of 46 women and their babies, hypovitaminemia, as defined, was found for pyridoxine, folate, vitamin B₁₂

and thiamin. Maternal and cord blood levels are compared for hypovitaminemic and normovitaminemic groups. The claim that cord blood levels for these four vitamins are lower in those babies born to hypovitaminemic mothers when compared with normal is not very obviously substantiated from the data as graphically presented.

Cord levels derived from data where no vitamins were given to the mother are generally higher than those pertaining in the maternal circulation. This is especially so in the case of riboflavin and pantothenate. Sequential data from successive deliveries indicates that, after injection, peak vitamin levels are mostly found coincidentally in both mother and fetus at one hour. Pantothenate peaks at two hours and cord riboflavin at two hours, i.e. an hour later than the maternal peak. It is claimed that vitamin C peaks at 30 minutes in both mother and baby but no observations before one hour are recorded. Cord levels are higher than maternal levels at all time intervals for thiamin, biotin, vitamin C, riboflavin, niacinamide and pantothenate but not for folate, pyridoxine and vitamin B₁₂. Thus although there may be placental transfer mechanisms favoring the fetus for some, this is not true for all the vitamins. The data suggest that such mechanisms may exist but could also be explained by different relative degrees of binding in serum or clearance by the fetal or maternal tissues.

General criticism must be made concerning the presentation of the data in this paper. For example, no standard deviation for each group of serial observations is given. The conclusions drawn are not always

substantiated by the data, numbers are small and the ensuing speculation goes farther than the facts might indicate. □

1. H. A. Kaminetzky, A. Langer, H. Baker, O. Frank, A. D. Thomson, E. D. Munves, A. Oppen, F. C. Behrle and B. Glista: The Effect of Nutrition in Teen-Age Gravidas on Pregnancy and the Status of the Neonate. I. A Nutritional Profile. *Am. J. Obstet. Gynec.* 115: 639-646, 1973
2. H. A. Kaminetzky, H. Baker, O. Frank and A. Langer: The Effects of Intravenously Administered Water-Soluble Vitamins During Labor in Normovitaminemic and Hypovitaminemic Gravidas on Maternal and Neonatal Blood Vitamin Levels at Delivery. *Am. J. Obstet. Gynec.* 120: 697-703, 1974
3. H. Baker, A. D. Thomson, O. Frank and C. M. Leevy: Absorption and Passage of Fat- and Water-Soluble Thiamin Derivatives into Erythrocytes and Cerebrospinal Fluid of Man. *Am. J. Clin. Nutrition* 27: 676-680, 1974
4. C. M. Leevy, L. Cardi, O. Frank, R. Gellene and H. Baker: Incidence and Significance of Hypovitaminemia in a Randomly Selected Municipal Hospital Population. *Am. J. Clin. Nutrition* 17: 259-271, 1965

THE ENDOCRINOLOGY OF ADULT PROTEIN-CALORIE MALNUTRITION

Adults with protein-calorie malnutrition have metabolic and endocrine changes similar to those seen in children. Thyroid and adrenocortical changes have been studied in detail and shown to be more complicated than had been appreciated previously.

Key Words: adult protein-calorie malnutrition, amino acids, thyroid hormones, growth hormone, adrenal cortex, cortisol, ACTH

Protein-calorie malnutrition (PCM) has been less intensively studied in adults than in children partly because of a lower incidence and partly because of preoccupation by the investigator with the effects of PCM on the developing individual. PCM in the adult is not rare, however, and is of special interest because comparison of the sick with the recovered patient is not complicated by the resumption of growth. Furthermore the larger size and cooperation of the adult make sophisticated investigations easier to perform and minimize ethical problems. Smith and his colleagues recently made a number of studies on Bengali adults with PCM, paying particular attention to endocrine changes.

The subjects were mainly youths and men with severe PCM who were selected from refugee camps, out-patient clinics and refugee camps in and near Calcutta. Clinical criteria for selection were the signs of severe PCM and the absence of other important disease such as tuberculosis. The patients were admitted to the hospital where they were initially fed a predomi-

nantly rice diet containing approximately 600 kcal and 12 g protein per day while intercurrent infections and infestations were treated and baseline measurements made. After completion of the initial studies, which lasted up to two weeks, the diet was changed to one containing approximately 3000 kcal and 150 g protein. The extra protein was derived mainly from milk, fish and eggs. When the subjects regained clinical normality, which took from two to four months, the tests were repeated.

The first study¹ concentrated on nitrogen and amino acid metabolism. On admission there was the characteristic reduction in body weight which was 72 percent of that attained on recovery. This was coupled with reduction in midarm cross-sectional area by 40 percent and urinary creatinine excretion by 58 percent. This loss of muscle mass coupled with edema and hypoalbuminemia led the authors to conclude that virtually all their patients had marasmic-kwashiorkor. In this study a specially low nitrogen intake of 2 g per day was given initially. Three to 14 days after admission urinary nitrogen excretion averaged 3 g per day. By theoretical cal-

culuation of stool and skin losses a figure for a negative nitrogen balance of 2.4 g per day was also achieved. After recovery the intake was 25 g per day, the urinary loss 13 g per day resulting in a net positive balance of 6.6 g per day. Recovery was associated with a rise in mean serum urea nitrogen from 8.8 to 15.8 mg per 100 ml.

Plasma levels of individual essential amino acids were all reduced on admission when compared with normal American men studied by the same workers. Values rose with refeeding and at the second test only the plasma methionine level was significantly reduced among the essential amino acids. Nonessential amino acids showed a different pattern, glycine levels being high and cystine, tyrosine, arginine, histidine and α -aminobutyrate being low on admission. Refeeding caused a general rise in plasma nonessential amino acids with the exception of glycine, and most became significantly higher than those of the American controls. Alpha-aminobutyrate was the most obvious exception, remaining significantly subnormal. Simultaneous forearm arterial and venous plasma from fasted patients was analyzed to estimate the net release or uptake of amino acids by forearm tissues. No change was noted between the malnourished and recovered states but comparison of either with normal American subjects revealed that the negative arteriovenous difference for alanine, threonine, histidine, lysine and proline was significantly less in the patients indicating a smaller release of these amino acids in PCM. In three subjects brachial artery blood flow was measured. This was grossly reduced on admission being 21 ml per minute compared with 64 ml per minute in the Americans and on recovery rose to 112 ml per minute. The significance of this apparent hyperemia is doubtful, however, because of the small number of observations. These results are in general what might have been expected by extrapolation from earlier work in infantile PCM but contrast with the findings in starved obese Americans.² They serve to document the patients clinically and in some respects

metabolically for the associated endocrine investigations.

Growth hormone (GH) secretion was studied in three ways on successive days via an indwelling plastic cannula.³ All doses of infusate were calculated as a function of ideal body weight. On the first day 0.5 g glucose per kilogram was infused over three minutes, on the second 0.5 g arginine per kilogram over 30 minutes and on the third, 50 g casein hydrolysate over two to five hours. The mean fasting plasma GH based on nine samples from each patient was 19.6 ± 3.5 ng per milliliter on initial study and 3.8 ± 0.5 ng per milliliter on recovery. The glucose infusion caused a rapid, significant fall in plasma GH of 5 ng per milliliter at three minutes which returned to the baseline by 30 minutes and subsequently rose in the hour thereafter to peak at twice fasting levels at 60 minutes. When the same test was performed on refed patients no change in plasma GH occurred.

Arginine infusion caused a rise in plasma GH both on admission and after recovery with no significant difference between the two except for the 90 minute postinfusion sample which was 16 ng per milliliter above basal in malnourished patients and similar to the peak response at 60 minutes, whereas on recovery the 90 minute sample was falling toward the fasting value. Casein hydrolysate produced a large but variable rise in plasma GH in the malnourished subjects which persisted for six hours and a much smaller but qualitatively similar response following recovery. The variation in individual responses may have been related to the wide time range for the infusion. The raised fasting plasma GH levels at the time of initial study were correlated with the plasma concentrations of amino acids and other metabolites but the only significant association found was a positive correlation between GH and urea nitrogen.

The fasting plasma GH levels in adults mimic those in infants with PCM closely.⁴ What may be of even greater interest, the response of the adult with PCM to intravenous glucose is similar to that of the normal newborn.⁵ The response of the

adult to intravenous arginine contrasts with that of infants in whom no rise in plasma GH occurs.^{6,7} There is also a contrast in response to casein hydrolysate in adults with the production of a fall in plasma GH in infants with PCM by a mixture of essential amino acids.⁸ This last difference may be related to the route of administration, however, since the infants were infused via a nasogastric tube.

Thyroid function was assessed⁹ by measuring plasma thyrotropin (TSH), thyroxine (T_4) and tri-iodothyronine (T_3) by radioimmunoassay, free T_4 and T_3 by equilibrium analysis and thyroxine binding globulin by competitive ligand binding assay. The mean plasma T_4 of the untreated patients was 8.2 μg per 100 ml which did not differ significantly from the value on recovery of 7.7 μg per 100 ml. The dialyzable fraction before treatment was 0.048 percent which was nearly twice the value in the same patients following recovery, 0.029 percent. This resulted in the mean free T_4 concentration being 3.8 ng per 100 ml on admission and 2.2 ng per 100 ml after refeeding. In contrast to these results the total plasma T_3 was 21 ng per 100 ml before treatment and 96 ng per 100 ml afterwards. As with T_4 the dialyzable fraction of T_3 was higher before treatment but not so much as to offset the difference in total T_3 concentration with the result that mean plasma free T_3 before treatment was 94 pg per 100 ml and afterwards 303 pg per 100 ml. In comparison with normal American adults the free T_4 of the patients was abnormally high before treatment and abnormally low after, whereas T_3 was low before and normal after.

Plasma TSH and thyroid binding globulin levels did not change with treatment and both were raised by normal American adult standards. The disturbance of thyroid function in PCM illustrated by these results is more profound and complicated than had hitherto been appreciated.⁴ The decrease in plasma albumin and thyroxine-binding prealbumin, both of which are capable of carrying T_3 , is inadequate to explain the large fall in plasma concentration of this hormone. Interference with

binding of T_3 cannot be invoked since bound T_4 was present in normal concentrations and free T_3 was reduced as well as the bound fraction. The question of alteration in peripheral metabolism of T_4 and T_3 seems a more promising possibility to explain the levels found initially. Degradation rates of either hormone have not been measured and will be contributory, but the most likely possibility seems to be that there is a failure of extra thyroidal deiodination of T_4 to T_3 . This concept has important clinical implications as the physiological actions of the two thyroid hormones are thought to differ; T_3 is concerned more with thermogenesis and T_4 is concerned with general metabolic activity.¹⁰ Again the changes in adult PCM may be recapitulating those in the normal neonate who also has a low serum T_3 and high T_4 .¹¹

The study of adrenocortical function in these patients also proved rewarding.¹² At the time of admission blood was collected at 0900 and 1300 for plasma ACTH and cortisol measurements and 24-hour urine collections were assayed for free cortisol, 17-hydroxycorticosteroids (17-OHCS) and 17-ketosteroids (17-KS). The metabolic clearance rate of cortisol was measured by infusion of tritiated cortisol. The integrity of the pituitary adrenal axis was studied using metyrapone, dexamethasone and ACTH.

The basal plasma cortisol levels at both 0900 and 1300 were raised before treatment in comparison to the refed state. The urinary free cortisol excretion was the same before and after treatment when expressed as total output, but when correction was made for the reduction in creatinine clearance in PCM the pretreatment free cortisol excretion was raised. The authors deduce from this that circulating free cortisol levels are raised in PCM. No significant difference could be demonstrated in plasma ACTH levels before and after refeeding, however. Urinary 17-OHCS and 17-KS were markedly reduced before treatment.

The mean clearance rate of cortisol at the time of initial study was approximately half that after treatment when the rate was

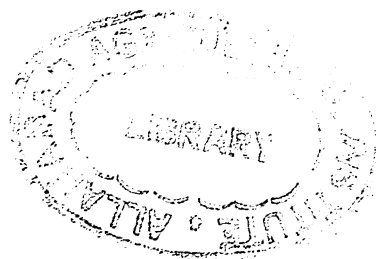
similar to that of normal Americans. The cortisol production rate did not differ significantly before and after refeeding. Plasma ACTH levels rose in response to metyrapone in a manner similar to that of normal Americans. ACTH produced a brisk and similar rise in plasma cortisol both before and after treatment. Dexamethasone, on the other hand, failed to suppress plasma cortisol in the patients at the time of initial study, but a normal response was obtained after they had recovered from PCM.

The results of these investigations are very similar to earlier studies on infants with PCM.^{13,14} The pathophysiology of cortisol secretion in PCM at both ages seems to have two important facets. The metabolism of cortisol is impaired resulting in a reduction in the mean clearance rate and in urinary 17-OHCS excretion together with a raised total and free plasma cortisol level in the face of reduced production rate. The other feature is that, although integrity of the adrenal response to ACTH is preserved, the other half of the feedback loop is set abnormally insofar as dexamethasone does not suppress plasma cortisol levels. This is probably the consequence of continued ACTH secretion despite adequate circulating levels of glucocorticoids. The possibility that a hypothalamic drive is overriding the potential glucocorticoid inhibition must be considered.

It is interesting in considering this group of papers to see how closely PCM in adult life mimics PCM in childhood. The work of Smith and his co-workers is helpful in documenting this fact, especially in areas where they have been able to investigate in more detail than might have been possible had their patients been infants. Their measurements of metabolites and polypeptide hormones essentially recapitulated what has already been described in younger patients. The observations on the thyroid hormones and cortisol on the other hand added new depth to our understanding of PCM and pose questions which may be of general physiological relevance. □

1. S. R. Smith, T. Pozefsky and M. K. Chhetri: Nitrogen and Amino Acid Metabolism in Adults with Protein-Calorie Malnutrition. *Metabolism* 23:603-618, 1974
2. P. Felig, O. E. Owen, J. Wahren and G. F. Cahill: Amino Acid Metabolism during Prolonged Starvation. *J. Clin. Invest.* 48: 584-594, 1969
3. S. R. Smith, P. J. Edgar, T. Pozefsky, M. K. Chhetri and T. E. Prout: Growth Hormone in Adults with Protein-Calorie Malnutrition. *J. Clin. Endocrinol. Metab.* 39: 53-62, 1974
4. Endocrine Adaptation to Malnutrition. *Nutrition Reviews* 30: 103-106, 1972
5. P. Stubbe, J. Leitis, W. Leppla and H. Wolf: Provocation of Growth Hormone and Insulin Secretion in Full-Term Infants Small for Gestational Age. *Pediat. Res.* 7: 58, 1973
6. F. Beas, I. Contreras, A. Maccioni and S. Arenas: Growth Hormone in Infant Malnutrition: The Arginine Test in Marsamus and Kwashiorkor. *Brit. J. Nutrition* 26: 169-175, 1971
7. G. G. Graham, A. Cordano, R. M. Blizzard and D. B. Cheek: Infantile Malnutrition. Changes in Body Composition during Rehabilitation. *Pediat. Res.* 3: 579-589, 1969
8. R. D. G. Milner: Metabolic and Hormonal Responses to Oral Amino Acids in Infantile Malnutrition. *Arch. Dis. Child.* 46: 301-305, 1971
9. I. J. Chopra and S. R. Smith: Circulating Thyroid Hormones and Thyrotropin in Adult Patients with Protein-Calorie Malnutrition. *J. Clin. Endocrinol. Metab.* 40: 221-227, 1975
10. P. N. Nathanielsz: Thyroid Function in the Fetus and Newborn Mammal. *Brit. Med. Bull.* 31: 51-56, 1975
11. J. Abuid, D. A. Stinson and P. R. Larsen: Serum Triiodothyronine and Thyroxine in the Neonate and the Acute Increases in These Hormones Following Delivery. *J. Clin. Invest.* 52: 1195-1199, 1973
12. S. R. Smith, T. Bledsoe and M. K. Chhetri: Cortisol Metabolism and the Pituitary-Adrenal Axis in Adults with Protein-Calorie Malnutrition. *J. Clin. Endocrinol. Metab.* 40: 43-52, 1975
13. G. A. O. Alleyne and V. H. Young: Adrenocortical Function in Children with Severe Protein-Calorie Malnutrition. *Clin. Sci.* 33: 189-200, 1967
14. P. J. Leonard and K. M. MacWilliam: Cortisol Binding in the Serum in Kwashiorkor. *J. Endocrinol.* 29: 273-276, 1964

NUTRITION CLASSICS



THE JOURNAL OF NUTRITION

VOLUME 56, NUMBER 3

July 11, 1955

Pages 371-386

STUDIES OF NIACIN REQUIREMENT IN MAN

II. REQUIREMENT ON WHEAT AND CORN DIETS LOW IN TRYPTOPHAN

GRACE A. GOLDSMITH, HAROLD L. ROSENTHAL, JANIS
GIBBENS AND WALTER G. UNGLAUB

*Nutritional Research Laboratory, Departments of Medicine and Biochemistry,
Tulane University School of Medicine and the Charity Hospital of
Louisiana at New Orleans, Louisiana*

(Received for publication January 24, 1955)

The niacin requirement of humans can be determined only in relation to the tryptophan content of the diet since it has been shown that the amino acid tryptophan is converted to niacin compounds in man as well as in many other species (Sarett and Goldsmith, '47, '49; Perlzweig et al., '47). Two basic diets have been devised for investigating niacin requirement, one high in corn, the other high in wheat. Each diet furnishes approximately 200 mg of

tryptophan which should supply little more than the minimum requirement for this amino acid (Rose, '49). In a previous study (Goldsmith et al., '52) each of three subjects who received the "corn" diet, which furnished approximately 4.7 mg of niacin and 190 mg of tryptophan for more than 50 days developed pellagra. On the other hand, no signs of niacin deficiency were observed in one subject who received the "corn" diet supplemented with 2 mg of niacinamide daily for 122 days, nor in a second subject who received a "wheat" diet, which furnished approximately 5.7 mg of niacin and 230 mg of tryptophan, for 95 days.

• • •

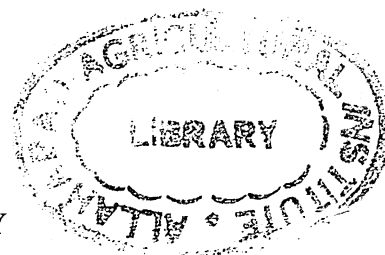
In this report, clinical findings and the urinary excretion of niacin and tryptophan metabolites during the "wheat" regimen will be discussed, and compared with those observed previously when the "corn" diet was administered. In addition, data obtained in 6 subjects who received the "corn" diet supplemented with several levels of niacinamide will be presented.

• • •

DISCUSSION

The association of corn diets with pellagra has been recognized for centuries. Possible explanations for this association include (1) the low tryptophan content of corn, (2) the presence of "bound" niacin in corn which is unavailable to the body, (3) imbalance of amino acids in diets which contain large quantities of corn products and (4) a "toxic" or inhibitory factor in corn.

• • •



SUMMARY

Three subjects were maintained for 90 to 105 days on a "wheat" diet which furnished approximately 5 mg of niacin and 200 mg of tryptophan daily. One subject developed typical niacin deficiency beginning 80 days after the diet was instituted, a second developed amenorrhea, herpes of the lip and slight redness of the tongue papillae, and a third showed lassitude and depression as the only clinical findings. In contrast to this, each of three subjects previously maintained on a "corn" diet of comparable niacin and tryptophan content showed the characteristic clinical picture of niacin deficiency after about 50 days of the experimental period. Excretion of N^1 -methylnicotinamide decreased to lower levels within a shorter period of time, and tryptophan excretion was slightly lower during the corn than during the wheat regimen.

The time at which pellagra developed, and the severity of the deficiency, seemed to be related to the intake of niacin and tryptophan per unit of body size with both the "wheat" and "corn" diets. However, this relationship may not completely explain differences in clinical and laboratory findings between the two regimens. The low tryptophan content of corn may not be the sole explanation of the pellagrogenic effect of this cereal.

When the "corn" diet was supplemented with varying amounts of niacinamide a significant increase in excretion of niacin metabolites occurred when the intake approximated 8 to 10 mg daily. These data suggest that with the "corn" diet, which furnishes 200 mg of tryptophan daily, body niacin stores approach adequacy when the diet supplies 8 to 10 mg of niacin.

BRAIN AND MYOCARDIAL LESIONS IN COPPER-DEFICIENT YOUNG RATS

When copper deficiency was produced in young rats by feeding their dams a low copper diet through gestation and pregnancy, the offspring showed changes in myelination and in the cerebellar levels of copper-containing enzymes. Myocardial damage, with death from heart failure, also appeared but the myocardial lesions were not accompanied by vascular lesions.

Key Words: copper deficiency, elastin, enzymes, brain lesions, cytochrome oxidase

In addition to the long-established requirement for copper in hematopoiesis, recent work demonstrated another function of copper in the vascular system. This is the requirement of copper for elastin formation.¹ Elastin, a structural protein occurring in aorta and tendons, is the basis of the elasticity of these organs. In copper-deficient chicks, the amount of elastin in the aorta is reduced. The decrease in elastin appears to result from decreased oxidative deamination of lysine. This is required for formation of desmosine, a cross-linked structure formed by the condensation of four lysine residues.¹ The enzyme, lysyl oxidase, appears to be involved in the oxidative deamination of the epsilon-amino group.² The activity of this enzyme, which may require copper, is reduced in the aorta and in the liver in copper deficiency. Addition of purified preparations of the enzyme to aortic tissues from copper-deficient rats increases conversion of lysine to desmosine.¹

The involvement of copper in the development of the nervous system is also well established in animal nutrition.³ In grazing animals, copper deficiency results in the condition called "enzootic neonatal ataxia". Neurological abnormalities have been observed in newborn guinea pigs and rats. These abnormalities, which depend upon the degree of copper deficiency, include neuronal necrosis in lambs, cerebellar

defects with delayed myelination in guinea pigs and necrotic neural tissue in rats. The cerebellum is especially susceptible to undernutrition because of postnatal interneuron and neuroglial proliferations.

Recent experimental work in both of these areas showed that brain lesions in the copper-deficient rat appear similar to some of the symptoms of Menkes' disease in children.⁴ Copper deficiency can also affect the myocardium itself, independent of the arterial damage caused by the deficiency.⁵ Menkes'⁶ disease (the steely-hair syndrome) in children is a sex-linked disorder which results in cerebral and cerebellar degeneration, scorbutic-like changes in bone and a change in hair texture. This condition appears to involve a defect in copper absorption since the reduced serum copper can be increased by high doses of oral copper supplements.⁷

In experiments testing the copper-deficient neonatal rats as a possible model for studying the neuropathology of Menkes' disease, weanling female rats were fed a low-copper diet (0.3 μ g copper per gram) for nine weeks, then bred. During gestation, they were fed on a diet containing 2 μ g copper per gram. After the birth of the young (eight pups allotted per dam), the dams were refed the low copper diet, and the young were continued on this diet at weaning. In the control experiment, female rats were fed a diet supplying 18 μ g per gram from the eighth day of gestation through lactation (eight pups allotted per

dam). The young were continued on this diet after weaning. The diet contained 25 percent casein, 5 percent fat with sucrose as the carbohydrate. The casein was washed with EDTA to reduce the copper content, and the drinking water was triple distilled.

The young female rats fed the copper-deficient diet appeared hyperactive when handled. Their growth was quite irregular; three of 18 rats died before parturition and three more died after parturition. These rats showed cardiac hypertrophy, softening of the bones and loss of surface vascularity of the cerebral hemispheres. The copper-deficient pups were smaller at birth and at all subsequent stages than the control pups. The deficient pups weighed 43 g at 22 days and 60 g at 28 days, in comparison with 66 g and 100 g for the controls at the same age. The brain and cerebellar weights of the deficient pups, however, were not significantly smaller, except at weaning. No other external symptoms appeared, except for the coat, which was thin and unkempt. (Presumably, the rats were of an albino strain since achromotrichia was not mentioned in the deficient rats.)

Analysis of the brains (minus cerebella) for copper showed that the concentration (micrograms per gram of wet tissue) in the controls tripled from five to 15 days of age (from ca. 0.6 to 1.5 μg per gram), then rose to 2.0 μg per gram at 24 days, with no additional increase at 28 days. In contrast, the concentration in the deficient rats was ca. 0.4 μg per gram at three days, 0.6 μg per gram at 12 days and 0.4 μg per gram at 28 days. Evidence for retardation in cerebellar development was a lower concentration of the enzyme, 2', 3'-cyclic nucleotide 3'-phosphohydrolase, which is used as an index of myelin formation in the central nervous system. In both groups, the activity of this enzyme increased from 12 to 28 days of age, but the activity remained significantly lower at all times in the deficient rats.

The enzymes, cytochrome oxidase and superoxide dismutase, are copper metalloproteins. The activities of both enzymes in the cerebellum were reduced in the

copper-deficient rats up to 28 days of age. At this time, the level of cytochrome oxidase in the controls was seven times higher than in the deficient pups. The levels of superoxide dismutase were the same in the deficient and control pups at 16 days of age, but after this time, the activity was significantly lower in the deficient group.

The norepinephrine content of the brain (without cerebellum) was measured as an index of the activity of the enzyme, dopamine-beta-hydroxylase, which is also a copper metalloprotein. In the controls, the concentration (nmoles per gram of wet tissue) increased from 0.7 at 12 days to 2.0 at 22 days, with no additional changes to 28 days. In the deficient group, the concentration increased to ca. 1.4 μmoles per gram at 22 days, with no change to 28 days.

Cardiac hypertrophy is a well-established symptom in copper-deficient rats and pigs.¹ Death in copper-deficient animals has previously been attributed to heart failure, but there were no reports of specific myocardial defects which were independent of previously established vascular disease.^{8,9} The cardiac hypertrophy was originally considered a compensation for the anemia of copper deficiency and/or the decrease in mitochondrial cytochrome oxidase, since it is a copper-containing enzyme. Later work showed that the heart enlargement in rats preceded the appearance of anemia and the decreased myocardial cytochrome oxidase activity.

Kelly, Kesterson and Carlton⁵ produced degenerative cardiac disease in young rats which were the offspring of dams fed a copper-deficient diet. In these experiments female rats were fed the experimental diets from three weeks of age through lactation. They were bred at 17 weeks. The offspring were then fed the same diets as their dams. The basal diet contained 20 percent casein with 5 or 15 percent fat and sucrose plus starch as the carbohydrate sources. The copper-deficient diet contained 0.9 μg copper per gram and the copper-supplemented control diet contained 8 μg copper per gram.

Deaths from heart disease occurred suddenly without previous symptoms in one or more pups in the deficient litters. Sudden death could be brought on by application of pressure to the thorax. Mortality of this type was highest between 40 to 50 days of age and did not appear after 60 days. The hearts of the deficient young were enlarged and pale. Other vascular lesions were not apparent so that the primary effect of copper deficiency was on the myocardium. The heart-body weight ratios at 27 days of age were 0.88 for the deficient rats and 0.49 for the controls. The ratio was 0.60 in rats transferred from the deficient to the control diet for one week. At weaning, the deficient offspring were somewhat anemic, but the hemoglobin and packed cell volumes again became normal after the early rapid growth stage when the cardiac abnormalities had appeared.

All areas of the heart showed histochemical evidence of reduced cytochrome oxidase activity, even in hearts which were apparently normal in outward appearance. Decreased staining for cytochrome oxidase also occurred in all areas of the brains from these animals, especially in the cerebral cortex, basal ganglia and thalamic regions, in agreement with the location of histopathological lesions described in earlier reports. The cytochrome oxidase levels in both heart and brain were nearly normal in deficient rats fed the control diet for 20 days.

Kelly and co-workers noted that the lack of development of degenerative cardiac lesions in other studies of copper deficiency may reflect the dietary copper level. In the present experiments, the level of 0.9 μg per gram was somewhat higher than that used by others and permitted normal growth and parturition in the first generation, but not in the second.

In addition to differences in copper level, studies of copper deficiency may be complicated by differences in age of the rats and by the use of milk diets to produce copper deficiency.¹⁰ With milk diets, the growth of even the copper-supplemented

rats may be poor, and these animals may show abnormalities when compared with those fed a chow diet. Consequently, the pattern of symptoms in this case would be a combination of copper deficiency and other abnormalities produced by a milk diet, including diarrhea and cecal hemorrhage, which might result from the high lactose intake with milk diets.¹⁰ The results of copper deficiency studies might also be complicated by the dietary zinc level which can affect copper utilization, perhaps by interfering with absorption.^{11,12}

The knowledge of the role of copper in the nervous and cardiovascular systems is needed for understanding whether pathological conditions in these tissues may be related to or affected by dietary factors. Hopefully, copper-deficient rats will be a useful model for understanding and preventing the pathological changes in conditions such as Menkes' disease. \square

1. C. H. Hill: A Role of Copper in Elastin Formation. *Nutrition Reviews* 27: 99-100, 1969
2. R. B. Rucker and W. Goettlich-Riemann: Isolation and Properties of Soluble Elastin from Copper-Deficient Chicks. *J. Nutrition* 102: 563-570, 1972
3. *Trace Elements in Human and Animal Nutrition* by E. J. Underwood. Third edition, pp. 57-115. Academic Press, New York, 1971
4. J. R. Prohaska and W. W. Wells: Copper Deficiency in the Developing Rat Brain: A Possible Model for Menkes' Steely-Hair Disease. *J. Neurochem.* 23: 91-98, 1974
5. W. A. Kelly, J. W. Kesterson and W. W. Carlton: Myocardial Lesions in the Offspring of Female Rats Fed a Copper Deficient Diet. *Exp. Molec. Path.* 20: 40-56, 1974
6. Menkes' Kinky Hair Syndrome. *Nutrition Reviews* 31: 17, 1973
7. Copper Metabolism in the Steely-Hair Syndrome. *Nutrition Reviews* 33: 189, 1975
8. C. E. Hunt and W. W. Carlton: Cardiovascular Lesions Associated with Experimental Copper Deficiency in the Rabbit. *J. Nutrition* 87: 385-393, 1965

9. G. S. Shields, W. F. Coulson, D. A. Kimball, W. H. Carnes, G. E. Cartwright and M. M. Wintrobe: Studies on Copper Metabolism. XXXII. Cardiovascular Lesions in Copper-Deficient Swine. *Am. J. Path.* 41: 603-617, 1962
10. G. A. Hall and J. McC. Howell: Lesions Produced by Copper Deficiency in Neonate and Older Rats. *Brit. J. Nutrition* 29: 95-104, 1973
11. L. Murthy, L. M. Klevay and H. G. Petering: Interrelationships of Zinc and Copper Nutrition in the Rat. *J. Nutrition* 104: 1458-1465, 1974
12. B. Alfaro and F. W. Heaton: Relationships Between Copper, Zinc and Iron in the Plasma, Soft Tissues and Skeleton of the Rat During Cu Deficiency. *Brit. J. Nutrition* 29: 73-85, 1973

New

Unique

INDEX OF NUTRITION EDUCATION MATERIALS

The Index of Nutrition Education Materials has been prepared by the Office of Education of the Nutrition Foundation to assist teachers and the general public in acquiring useful teaching aids and educational materials.

The Index lists more than 1,000 booklets, pamphlets, audio-visual aids and lists of recommended books available from federal agencies, professional societies, and educational organizations or foundations. Other sources for education material are included.

Gives addresses of important local contacts and sources in each state.

Source, subject matter, readership level and description are given for each publication in color-coded sections for easy reference. \$5.00 postpaid

Order from:

OFFICE OF EDUCATION
THE NUTRITION FOUNDATION
888 Seventeenth Street, N.W.
Washington, D. C. 20006

AN ACCESSIBLE ARCHIVE OF HUMAN EXPERIENCE

A unique, historical collection of papers, correspondence and other memorabilia, covering a sixty year period of the conquest of pellagra was recently donated by Dr. William Henry Sebrell, Jr. to the Vanderbilt Medical Center library. Dr. William J. Darby accepted this gift on behalf of the university during the Centennial Celebration of the founding of Vanderbilt University School of Medicine.

I am privileged to greet this Centennial audience on behalf of one of our warm friends, one of the nation's most distinguished nutrition scientists and research administrators, Dr. William Henry Sebrell, Jr. — who, due to illness, cannot be with us tonight. It is my pleasant task to express his high assessment of the leadership in nutrition, past and future, of our Medical Center, through his gift to us of a unique, selected collection of scientific papers, reprints, classical texts, translations, and correspondence pertaining to the work and research of the late Joseph Goldberger and Dr. Sebrell's own outstanding research. These cover a 60-year period of the conquest of a major disease — pellagra — of American origin, of worldwide importance, and of particular impact on the South.

This deficiency disease, which caused hundreds of thousands of deaths in the South, was eliminated by the work of Goldberger, Sebrell and their colleagues.

The world-wide distribution of pellagra in 1912 was depicted in a monograph by Stewart Roberts of Atlanta. The dedicatory statement in this 1912 book reads:

To that long line of physicians and scientists from Casals through Lombroso to Sambon, and those who shall come after them who have been and are and shall be students of pellagra

THIS VOLUME IS DEDICATED BY
THE AUTHOR

with the hope that the day is not far distant
when there shall arise from among them one

to whom shall be revealed with clear and certain proof the true cause of Mal de la Rosa (as it was initially called in Spain).

In 1914, Joseph Goldberger, a bacteriologist with the U.S. Public Health Service, was assigned the task of identifying the cause of the disease in the South. It was then thought to be of infectious origin. The victims suffered from the "3-D's" — Dermatitis, Diarrhea, Dementia — and several mental institutions were primarily devoted to the care of pellagrins.

Goldberger, in his now classic epidemiologic studies, noted the association of the disease with poor diet — the "3-M's" — Meal, Meat (fat back), Molasses, and with poverty. (One of the folk names for pellagra was "corn bread fever"). Further he observed that well fed persons did not contract the disease. He reproduced the condition in convicts in Mississippi by feeding them a deficient pellagrigenic diet. He, his wife, and 14 volunteer colleagues constituted a "filth squad" who ingested and were injected with various biological materials and/or excreta from patients, thus demonstrating the non-infectious nature of pellagra. In orphanages, prisons, and in mental institutions the therapeutic value of a good diet was demonstrated. Foods were assayed in man for their pellagra-curative properties, and for their pellagra-preventive action in an animal model, canine blacktongue.

Dr. Milton Terris, Professor of Preventive Medicine, New York Medical College, wrote in 1964 concerning Goldberger's work:

The pellagra studies provide a brilliant example of the use of epidemiologic reasoning — that is reasoning based on the behaviour of a disease in a population — to develop an etiologic hypothesis. Even more remarkable is the complete and incontrovertible demonstration, step by step, of the truth of that hypothesis. Finally the whole epidemiologic concept is presented; attention is focused not only on the dietary causes of pellagra but also on the underlying reasons for the dietary deficiencies. There emerges a thorough analysis of the economic and social basis of pellagra in the South, that is, of the total context in which pellagra took root and flourished.

These findings, controversial at the time — 1915-1918 — and even later, served as the basis for instituting world-wide control measures. Goldberger died in 1929. One indication of his position in American medicine is the naming of an award of the American Medical Association "The Goldberger Award in Clinical Nutrition." The first recipient of this award, financed by The Nutrition Foundation, fittingly was Dr. W. H. Sebrell, Jr. Two of these have come to Vanderbilt faculty members (Dr. John B. Youmans and myself). A fourth recipient is Dr. Cicely Williams, the distinguished, internationally known pediatrician, who is with us this evening.

Dr. Sebrell worked with Goldberger during the late 1920's and succeeded him as Director of the Public Health Service Hygienic Laboratory, which later evolved into the National Institutes of Health. He became Director of the National Institutes of Health during the period of accelerated growth of that great organization and, during his tenure, the Clinical Center was completed and opened.

The record of the "long line of physicians and scientists... students of pellagra... including the unfolding of the clear and certain proof [of] the true cause of Mal de la Rosa" is included in the remarkable collection that now becomes part of the permanent archives of our Medical Center as a result of Dr. Sebrell's generosity.

Study of this collection will reveal to students and scholars not only the details

of the conquest of a major disease, but also the intimate personal struggles produced by the inevitable tensions that develop between scientists during vigorous pursuit of crucial new information and its acceptance and application.

Re-examination of the Goldberger-Sebrell collection can contribute much to understanding critical issues facing medicine, science and society today.

To give us the benefit of understanding the etiology and to prevent pellagra, Goldberger assumed the risks inherent in the production of the disease in convicts, and the risk of trials with orphans, inmates of mental institutions, the displaced and the poor... in order to obtain "clear and certain proof of the true cause of" pellagra... among each of these most affected groups.

In his last public address in 1928 he stated:

Now it so happens that, conservatively estimated, there were some 120,000 people in the United States last year who suffered an attack of pellagra. One may ask, therefore, why, if the material is so simple, do so many people continue to be stricken with the disease? The answer lies in the fact... that the problem of pellagra is in main a problem of poverty.

Education of the people will help; but improvement in basic economic conditions alone can be expected to heal this festering ulcer of our people. This, obviously, cannot be accomplished in a day, but that day will be hastened by the co-operative action of all whose vision enables them to see the great social and economic advantage to be derived from the eradication of the disease...

This in 1928!

For those today puzzled by public controversies between individual members of the scientific community, it is perhaps revealing to read that two years later, in 1930, a prominent member of the medical community stated:

All the evidence of which I have personal knowledge, to which I am able to attach much weight, favors the opinion that it [pellagra] is due to an infection. I am content to remain with the minority who have not been

convinced by supposed proof of other causes, and still believe that a specific infection will be found to be the true cause . . .

The distinguished historian, Lynn White of the University of California, has modestly suggested that historians may have a contribution to make to technology assessment. I submit that a historical study of such valuable resources as comprised by this collection has much to contribute to assessment of medical ethics in relation to science and society today.

It is my earnest hope, Mr. Chancellor and Mr. Vice-Chancellor, that this gift, so fitting an example of an American achievement as to be described contemporarily by Professor Milton Rosenau, "the equal to any contribution to medical science made

in America . . .", that this gift will challenge all of us as we mark the Centennial of this great Medical School and enter the Bicentennial of our country. May this challenge be two-fold:

- 1) To examine the contributions and methods of medical science to society from a mature historical viewpoint;
- 2) To support and build our University Medical Center so that it becomes that which Carlyle identified

"The true University [as]
a Collection of Books"

— indeed, *an accessible archive of human experience* without which the creative scholarship that characterizes great universities cannot survive. □

Exchange of Personal Nutrition Collections

If one dates the science of nutrition from the discovery of oxygen and the process of oxidation, the discipline is just completing its second century. The enormously varied discoveries and advances of the twentieth century represent a major portion of the present knowledge of nutrition. Many of the outstanding contributors to this science are still living; others have but recently died.

Much is to be learned of the methods of science from the study of the collected materials with which a productive scholar surrounds himself and from his record of exchanges with fellow scientists, colleagues and others. The historian long has appreciated the value of retrospective studies. Often it is useful to examine early drafts of subsequently published papers, especially those reports which become epoch-making or represent scientific landmarks. Most of such records remain unpublished, and these studies are possible only as a result of preserving the papers of indi-

viduals in a manner that makes them accessible to others.

It is timely, therefore, for nutritionists to encourage the preservation of personal correspondence, papers and other memorabilia of colleagues whose careers have made an impact upon this science and its application for the betterment of mankind. Such collections appropriately can be housed in libraries of universities where there is vigorous interest in food and nutrition. The existence of these archives should be widely publicized in order that students of all ages may be aware of them.

In keeping with this philosophy, the gift by Dr. William Henry Sebrell, Jr. of the Goldberger-Sebrell Collection to the Vanderbilt University Medical School is an outstanding example of a generous action by a distinguished American nutritionist. Nutrition scientists everywhere will be grateful to him. It is hoped that his example will be followed by others who have the guardianship of valuable resource materials. It fur-

ther is hoped that libraries and other centers of scholarship will seek and appropriately preserve such collections.

To facilitate exchange of information pertaining to established collections, potential donors or recipients, The Nutrition Foundation is pleased to make its resources and services available. From time to time the location and nature of existing resources will be published and indexed in

NUTRITION REVIEWS. Correspondence relative to collections is invited in order that NUTRITION REVIEWS may publish an initial listing early in the bicentennial year. Please address inquiries to:

Accessible Archives
The Nutrition Foundation
489 Fifth Avenue
New York, New York 10017

JAN 6 1976

New from Academic Press

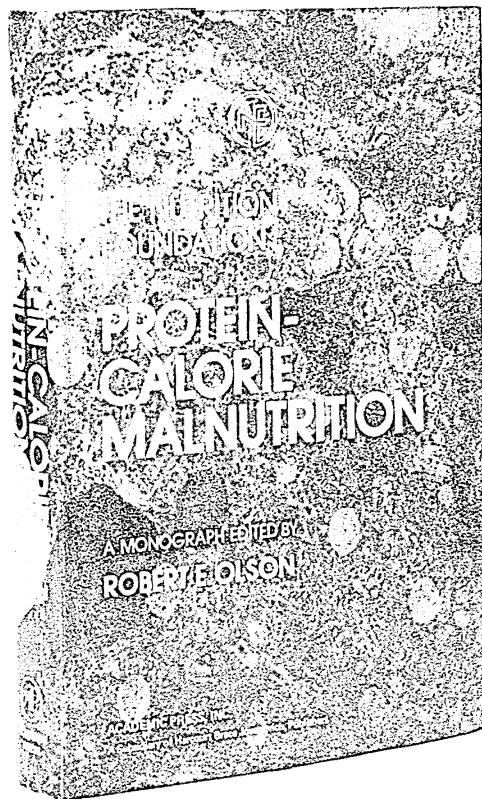
PROTEIN-CALORIE MALNUTRITION

A Nutrition Foundation Monograph

For physicians, nutritionists, public health practitioners, social workers, and medical students.

Covers all aspects of protein-calorie malnutrition (PCM), from molecular biology to epidemiology and prevention.

467 pages, \$29.50. Order from:
Academic Press, 111 Fifth Avenue,
New York, New York 10003
or your book supplier.



Grace A. Goldsmith, M.D.

April 8, 1904–April 28, 1975

Highly significant contributions to research, education, scholarship and service in nutrition, medicine and public health earned a permanent place among the leaders in American medicine for Dr. Grace A. Goldsmith.

Dr. Goldsmith earned her M.D. degree in Tulane University School of Medicine in 1932 and, following subsequent training at the Mayo Clinic, returned to Tulane as an instructor in medicine, subsequently to become a full professor of medicine in 1949. At Tulane she established a Nutrition and Metabolism Section in the Department of Medicine which justly became world famous, not only through its research contributions, but as an outstanding center for training of young scholars. In 1967, she assumed the Deanship of the Tulane University School of Public Health and Tropical Medicine and served in that office until 1973.

The first of Dr. Goldsmith's 189 scientific papers was published in 1934. In 1938, her paper on vitamin C deficiency appeared, the first in a long series of researches in the field of nutrition. Two years later she was an author, with A. T. Ogaard and D. F. Gowe, of a report entitled "Vitamin C Nutrition and Pellagra." Thus began her series of remarkable studies of pellagra, nicotinic acid, riboflavin, vitamin B complex deficiencies, folic acid and nutritional macrocytic anemia, tryptophan-niacin interrelationships, vitamin B₁₂ and intrinsic factor, dietary requirements of man, lipid metabolism and the influence of nicotinic acid on serum lipids, assessment of nutriture, the effectiveness of nutritional enrichment of cereals and the beneficial application of nutritional knowledge to the practice of medicine and public health.

It is in large measure due to Dr. Goldsmith's studies of the relationship of niacin and tryptophan that the quantitative aspects of our present estimation of their interrelationship in foodstuffs is founded. Observations made during the course of her experimental human studies of niacin deficiency and of riboflavin deficiency contributed greatly to the reliability of estimates of minimal requirements of these essential nutrients.

Dr. Goldsmith made the first report on the effectiveness of folic acid in the treatment of nutritional macrocytic anemia. The subsequent series of reports from her laboratory clarified many aspects of interrelationships between folic acid and vitamin B₁₂ in several clinical syndromes which respond in part or in toto to these nutrients. These reports also advanced understanding of the mechanism of the interaction between vitamin B₁₂ and the intrinsic factor.

Immediately prior to enrichment of flour in Newfoundland, Dr. Goldsmith directed a nutrition survey aimed at defining the nutriture with special reference to the B vitamins and iron of a population group on the west coast of this province. Four years later she directed a re-evaluation of this population and described the degree of improvement attributable to enrichment. From this point onward, Dr. Goldsmith's interest increasingly turned to the broad aspects of public health nutrition, although she maintained a productive clinical and laboratory investigative program.

In the course of her research career, Dr. Goldsmith worked with many young scientists and provided inspirational leadership and opportunities for their development. Among those who enjoyed these associa-

tions were Dr. Roy E. Butler, Dr. James G. Hamilton, Dr. O. Neal Miller, Dr. H. P. Sarett, Dr. Jack L. Smith and the late Dr. Walter G. Unglaub.

Dr. Goldsmith served with great distinction as a member of the Food and Nutrition Board, 1948-1969. She chaired the Committee on Dietary Allowances of the Food and Nutrition Board, 1950-1956, and served as Chairman of that Board for the decade, 1958-1968. She was a member of the Council on Foods and Nutrition of the American Medical Association during the two periods, 1953-1963 and 1965-1975, and served as Chairman, 1970-1972. She was President of the American Society for Clinical Nutrition, 1972-1973, and a member of several major editorial boards, among which were the American Journal of Clinical Nutrition, the Journal of Nutrition, Archives of Internal Medicine, Journal of American Medical Woman's Association and Physiological Reviews.

Her activities on advisory committees and councils included practically all major organizations, nationally and internationally, in the field of nutrition: U. S. Public Health, National Institutes of Health, Institute of Nutrition for Central America and Panama, Food and Agriculture Organization of the United Nations, U. S. Department of Agriculture, Massachusetts Institute of Technology's Department of Nutrition and Food Sciences, The Nutrition Foundation and the Study Commission on Dietetics of the American Dietetic Association.

Her list of deserved honors was indeed long and included the Goldberger Award in Clinical Nutrition of the American Medical Association (1964), Honorary membership in the American Dietetic Association

(1963), Osborne-Mendel Award of the American Institute of Nutrition (1959), Seal Harris Medal of the Southern Medical Association (1970) and Modern Medicine Award for Distinguished Achievement (1975). In 1974 she was awarded the Mastership of the American College of Physicians and became a Fellow of the American Institute of Nutrition. Dr. Goldsmith was to have received an honorary doctorate from the University of Wisconsin on May 17, 1975.

Dr. Goldsmith was selected by the honorary medical society, Alpha Omega Alpha, as one of the Leaders in American Medicine whose memoirs are recorded in a videotaped interview. This interview, made shortly before Dr. Goldsmith's terminal illness, provides a lasting audio-visual record of the charming, warm, vivacious personality whose scholarly contributions and educational achievements earned for her a major rank among medical leaders of our day.

Throughout her extraordinarily rich and active career, Grace Goldsmith maintained a continuing involvement in patient care and the practice of internal medicine. It is likely that her exceptional effectiveness as a clinical investigator and teacher stemmed in large measure from the insight and scope provided by her duties in the care of the sick and indigent.

It is fitting that the legacy of excellence exemplified by her career and influence are memorialized in the:

Grace A. Goldsmith Student Loan Fund
Tulane University School of Public
Health and Tropical Medicine
1430 Tulane Avenue
New Orleans, Louisiana 70112

Leaders in American Medicine

Alpha Omega Alpha, in collaboration with the National Library of Medicine/National Medical Audiovisual Center, has produced a series of videotaped interviews recording the memoirs of leaders in American medical science. The films are available as teaching aids, archival deposits, and historical resources.

Dr. Grace A. Goldsmith was interviewed last year in this series by William J. Darby, President of the Nutrition Foundation. The subject of their discussion is "Nutrition; Interrelationships of Niacin and Tryptophan; Vitamins of the B-Complex and Macrocytic Anemia; Clinical and Laboratory Tests for Evaluation of Nutritional Status; and Lipid Metabolism in Man."

The program has been made possible through a benefaction from Professor Beatrice C. Seegal and the late Professor David E. Seegal. The Seegals had become convinced that audiovisual histories offered a unique means of extending the educational reach of a great teacher far beyond his or her own institution. They felt that the concept of "oral history" should be modified and expanded by the adoption of the videotaped interview.

With the inspiration and firm backing of the Seegals, Alpha Omega Alpha approached the National Library of Medicine and received enthusiastic endorsement for the use of its staff and facilities at the National Medical Audiovisual Center in Atlanta, Georgia.

In accordance with the Seegals' original concept, the aim of the program is to avoid comprehensive oral histories. In fact the subject is requested not to recite a detailed account of his or her life. Instead, emphasis is placed on the key points in his academic career; significant influences by persons and events; his major contributions to medicine and medical science; and the ebb

and flow in his major discipline during his career. Given an eminent leader and perceptive interrogator, such films may be not only educational and archival deposits, but, as well, unique sources of inspiration for younger men and women in building their careers.

The autobiographical memoirs of eminent medical scientists and teachers on videotape are shown in the accompanying table.

Copies of the recordings in the Alpha Omega Alpha/National Library of Medicine film series are available as follows:

For motion pictures, available in black and white only, playable on standard 16mm motion picture projectors with optical sound, write to:

National Medical Audiovisual Center
(ANNEX)
Station K
Atlanta, Georgia 30324

These films are available on loan only for a period of about three weeks—they are not available for purchase or permanent loan. The order must be received in Atlanta three weeks before date of presentation. When ordering specify the date when the film will be shown.

For full-color television videotape cassettes, suitable for small audiences, send a blank 60-minute videocassette in the 3/4-U format to:

National Medical Audiovisual Center
Videotape Duplication Program
1600 Clifton Road, N.W.
Atlanta, Georgia 30333

This is the only format made available on videotape. NMAC does not service one-half inch requests. Please allow six weeks for filling of orders. These tapes become the property of the person placing the order.

ALPHA OMEGA ALPHA INTERVIEWS

Scientist	Title	Interviewer
Lowell T. Coggeshall, M.D.	Medical Education, Tropical Medicine	John Z. Bowers, M.D.
George W. Corner, M.D.	Anatomy, Physiology of Mammalian Reproductive System; Identification of Progesterone; History of Science	John Z. Bowers, M.D.
Martin M. Cummings, M.D.	_____	Peter D. Olch, M.D.
Lester R. Dragstedt, M.D., Ph.D.	Physiology of the Stomach, Pancreas, Parathyroid Glands; Pathogenesis of Peptic Ulcer; Intestinal Obstruction and Parathyroid Tetany; Introduction of Vagotomy in Treatment of Peptic Ulcer; Discovery of Lipocaic	John Landor, M.D.
John F. Enders, Ph.D.	Virus Disease of Man and Animal	Frederick C. Robbins, M.D.
George L. Engel, M.D.	Problems of Clinical and Psychosomatic Medicine	Sanford Meyerowitz, M.D.
Jacques Genest, M.D.	Human Arterial Hypertension; Relationship of Kidneys and Adrenals to Hypertension and Salt Regulation; Electrolytes and Renal Function	Vincent P. Dole, M.D.
Grace A. Goldsmith, M.D.	Nutrition; Interrelationships of Niacin and Tryptophan; Vitamins of the B-Complex and Macrocytic Anemia; Clinical and Laboratory Tests for Evaluation of Nutritional Status; Lipid Metabolism in Man	William J. Darby, M.D., Ph.D.
Tinsley R. Harrison, M.D.	Cardiovascular Disease	Arthur J. Merrill, M.D.
A. Baird Hastings, Ph.D.	Physiology of Fatigue; Acid-Base Balance; Salt and Water Exchange; Biological Oxidations; Intermediary Metabolism; Quantitative Histochemistry; Use of Isotopes as Biochemical Tracers; Clinical Chemistry	Peter D. Olch, M.D.
Emile Holman, M.D.	Cardiovascular Disease: Definitive Studies on Arterio-Venus Fistulae; Pathophysiology and Treatment of Constrictive Pericarditis	Peter D. Olch, M.D.
Charles B. Huggins, M.D.	Calcium Metabolism; Sex Hormones; Experimental Surgery; Enzymes of Blood; Bone Physiology	Leon O. Jacobson, M.D.
Walsh McDermott, M.D.	Public Health; Investigation and Research of Infectious Diseases; Chemotherapy of Tuberculosis	David E. Rogers, M.D.
Karl F. Meyer, M.D.	Experimental Typhoid Infections; Botulism; Brucellosis; Psittacosis; Encephalitides Leptospirosis; Sylvatic Plague; Plague Immunity	E. B. Shaw, M.D.
Leo G. Rigler, M.D.	Radiology, Roentgen Diagnosis	Harry Z. Mellins, M.D.
Helen B. Taussig, M.D.	Congenital Malformations of the Heart; Blue Babies	Helen S. Pittman, M.D.

Howard C. Taylor, Jr., M.D.	Cancer of Female Reproductive Organs; Physiology of Pregnancy	Allan C. Barnes, M.D.
Matthew Walker, M.D.	Experimental Peritonitis and Penicillin; Wound Healing; Streptomycin; Massive Intestinal Resections; Anti-Cancer Drugs in Treatment of Cancer; Prevention and Treatment of Metastases in Mice and Humans	Louis J. Bernard, M.D.
Owen H. Wangenstein, M.D.	Intestinal Obstruction; Etiology of Appendicitis; Peptic Ulcer Problem; Precursors of Visceral Cancer; Cancer Detection	K. Alvin Merendino, M.D.
Shields Warren, M.D.	Pathology of Diabetes Mellitus; Tumors and Tumor Metastases; Effects of Radiation on Normal and Neoplastic Cells and Mammals	John Z. Bowers, M.D.
Cecil J. Watson, M.D.	Liver and Biliary Tract Disease; Jaundice and Anemia; Urobilin and Porphyrin Metabolism	Robert B. Howard, M.D., Ph.D.
Joseph T. Wearn, M.D.	Physiology of the Kidney; Blood Vessels of the Heart; Circulation of Heart and Lungs; Thebesian Circulation; Heart Disease; Capillaries of the Lung; Medical Education	T. Hale Ham, M.D.
Maxwell M. Wintrobe, M.D.	Hematology; Clinical and Experimental Nutrition, Especially Nutritional De- ficiencies in Swine; Leukemia and Related Neoplastic Diseases	Alexander M. Schmidt, M.D.
W. Barry Wood, Jr., M.D.	Research in Etiology of Pneumonias; Phenomenon of Fever	Robert J. Glaser, M.D.

Committee on Nutritional Anthropology

A new professional group, the Committee on Nutritional Anthropology of the Society for Medical Anthropology, has been organized in order to enhance communication among nutritionists and anthropologists working on sociocultural aspects of diet and food as they relate to health.

Committee members receive a twice-yearly newsletter. The Committee meets annually at the time of the meetings of the American Anthropological Association. The next meeting will be December 4, 1975, at the Fairmont Hotel in San Francisco, California. The Committee will present its third annual symposium to the American Anthropological Association in San Francisco on December 6, 1975. For further information about the Committee contact: Dr. Norge W. Jerome, Department

of Human Ecology and Community Health, University of Kansas Medical Center, Kansas City, Kansas 66103, or Dr. Christine S. Wilson, Department of International Health, University of California Medical Center, San Francisco, California 94143. □

Meeting Announcement

A Symposium on the Myelomeningocele Patient—A Multidisciplinary Approach will be held March 11-13, 1976 at the University of Cincinnati Medical Center, Cincinnati, Ohio. For further information contact the Office of CONMED, Suite E251, 231 Bethesda Avenue, Cincinnati, Ohio 45229. □

New NAS/NRC Report on Program Planning

Nutrition and Fertility Interrelationships: Implications for Policy and Action. Published by Food and Nutrition Board, National Academy of Sciences/National Research Council, Washington, D.C. 20418. Pp.67. Price \$4.50.

This concise report identifies the common attributes of nutrition and family-planning programs, such as the use of related facilities to carry out services and the employment of trained health workers to administer varied services. Practical opportunities for program integration are discussed with regard to distribution of commodities, dissemination of educational services, collection of survey data, program evaluation, and multipurpose workers. The possible benefits to be derived from an integrated attack on malnutrition and high fertility are demonstrated. Among the advantages cited is the pooling of funds and other limited resources. The report recog-

nizes that certain aspects of family planning and nutrition cannot be, or should not be, integrated and that one service should never be contingent upon the acceptance of the other.

In addition to the medical and social bases for integrated programs, the report explores important issues of organization and administration and gives attention to policy and program development and the need for continued research on the interrelationships between nutrition and fertility.

"Nutrition and Fertility Interrelationships: Implications for Policy and Action" is intended for nutritionists; public health workers and officials; family planners; decision makers in existing programs, private agencies, and government; and those engaged in population studies. The domestic and international effects of a program such as the one presented in the report could be far-reaching. □

Recent Books

Peptide Transport in Protein Nutrition, D. M. Matthews and J. W. Payne, editors. Published by North-Holland Publishing Company. U. S. distributor: American Elsevier Publishing Company, Inc., 52 Vanderbilt Avenue, New York, New York 10017. Pp. 503. Price \$54.25.

A Study of Food Consumption by the Duplicate Portion Technique in a Sample of the Dalby Population. B. Borgström, A. Nordén, B. Åkesson and M. Jägerstad, Editors. Published by The Almqvist & Wiksell Periodical Company, Stockholm, Sweden. Pp. 98.

World Review of Nutrition and Dietetics. Volume 22. Edited by G. H. Bourne. Published by S. Karger AG, Basel, Switzerland, Pp. 346. Price \$72.25.

Protein Nutritional Quality of Foods and Feeds, Part 1, Assay Methods—Biological, Biochemical, and Chemical, M. Friedman, editor. Published by Marcel Dekker, Inc., 270 Madison Avenue, New York, New York 10016. Pp. 626. Price \$49.50.

Prevention of Microbial and Parasitic Hazards Associated with Processed Foods: A Guide for the Food Processor. Published by Food and Nutrition Board, National Academy of Sciences/National Research Council, Washington, D. C. 20418. Pp. 170. Price \$5.75.

Efficient Resource Use for Tropical Nutrition: Nigeria, by V. E. Smith. Published by Michigan State University Press, East Lansing, Michigan. Pp. 401. Price \$9.50

Childhood Obesity. Edited by Myron Winick. Volume III in the Wiley Series on Current Concepts in Nutrition. Published by John Wiley & Sons, Inc., 605 Third Avenue, New York, New York 10016. Pp. 189. Price \$16.95.

Subunit Enzymes: Biochemistry and Functions, K. E. Ebner, editor. Published by Marcel Dekker, Inc., 270 Madison Avenue, New York, New York 10016. Pp. 352. Price \$24.50.

Cobalamin: Biochemistry and Pathophysiology. Edited by Bernard M. Babior. Published by John Wiley & Sons, Inc., 605 Third Avenue, New York, New York 10016. Pp. 447. Price \$25.00.

Nutrition: An Integrated Approach, by R. L. Pike and M. L. Brown. Published by John Wiley & Sons, Inc., 605 Third Avenue, New York, New York 10016. Pp. 1082. Price \$18.95.

D-Xylose Approved as a Diagnostic Agent

FDA recently approved a New Drug Application for use of D-xylose . . . in evaluating intestinal absorption. Although this agent has been used for this purpose for many years, until recently no sponsor was willing to assume responsibility for submitting data for new drug approval and for marketing it as a properly labeled diagnostic agent.

D-xylose . . . is partially absorbed from the small intestine; only a relatively small fraction is metabolized and the remainder is excreted in the urine. Measurement of the amount of the compound excreted into the urine (or, in infants and young children, present in the blood) during a desig-

nated time interval thus permits an assessment of the efficiency of intestinal absorption.

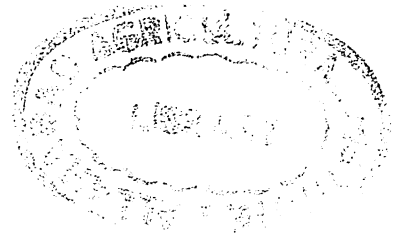
The sole use of D-xylose is as one of the diagnostic procedures for evaluating intestinal absorption and for the diagnosis of malabsorption states. Malabsorption may occur in any disease which affects the small bowel directly or indirectly, including such conditions as celiac disease, tropical or temperate sprue, Crohn's disease, immunoglobulin deficiency, pellagra, ascariasis, stagnant loop syndrome, radiation enteritis and surgical resection. □

Reprinted from the *FDA Drug Bulletin* 5:11, July-August, 1975.

Special Notice

All subscription inquiries, renewal requests and change of address notices for NUTRITION REVIEWS should be sent to:

The Nutrition Foundation
Office of Education
888 Seventeenth Street, N.W.
Washington, D. C. 20006



Dietary Fiber

by Albert I. Mendeloff, M.D., M.P.H.

17 APR 1976

In recent years attention has turned to items of the diet which have not occupied the center of the biochemical stage during the past 50 years. Among these may be mentioned items for which earlier methodological approaches have been undependable, such as the trace metals, and those for which even the definition has been elusive. Certainly among this latter group is what is commonly called dietary fiber.

Everyone has an idea of what fiber is, but there is no agreement about a quantitative approach to it as it exists in human food supplies. Previous tables of dietary fiber are based on methods developed by Einhof from 1806 to 1809, in which the fibrous residue left after sequential extraction with solvent, dilute aqueous acid and dilute alkali, represented the "indigestible matter." In very recent years a new approach to this obviously unsatisfactory methodology has been urged, but few advances have been made. As summarized by one team of leading workers¹ in this field, "the problems are:

- (a) conflicting concepts of what constitutes fiber;
- (b) the definition of lignin, cellulose and hemicellulose;

- (c) achieving separation of lignin from interfering matter;
- (d) the isolation of indigestible fiber and its relation to the true fiber of food; and
- (e) the failure of hemicellulose, cellulose and lignin to be biologically or chemically similar in different plant materials."

Energy Availability

Obvious interest in these aspects of ruminant nutrition has spilled over to human nutrition in recent years, with speculation as to whether "nondigestible" foodstuffs might actually result in energy availability, and particularly whether gut flora in the colon might change this material to produce colonic disease. Southgate² set up a comprehensive nomenclature to define the possible carbohydrates of the human diet, in which *crude fiber* includes cellulose and lignin together with variable amounts of other polysaccharides, *unavailable carbohydrate* refers to all plant polysaccharides not hydrolyzed by secretions of the human digestive tract and *dietary fiber* refers to unavailable carbohydrates and lignin, the latter being a phenylpropane derivative rather than a carbohydrate.

This nomenclature is an attempt to clarify the problem rather than to solve it. There is no question that appreciable quantities of unavailable carbohydrates disappear during their passage through the gut, but the best evidence available³ suggests that their contribution to the total energy intake of man on a mixed Western diet is less than 3 percent of the total energy of the diet. Current tables of dietary fiber

Dr. Mendeloff is Physician-in-Chief at the Sinai Hospital of Baltimore, Baltimore, Maryland 21215, and Professor of Medicine in the Gastrointestinal Division, Department of Medicine, Johns Hopkins University School of Medicine, Baltimore, Maryland 21215.

A limited number of reprints of this article may be obtained from the author. THERE ARE NO REPRINTS OF UNSIGNED REVIEWS.

content of foodstuffs obviously underestimate the fiber content, but we do not know by how much.

Digestive Tract Physiology

When we turn our attention to the effects of fiber on normal digestive tract physiology, we are on equally unsatisfactory ground, because we now have to study the physical properties of these widely varying materials, and at widely differing dosage levels. Furthermore, we have to realize that large intakes of such materials are customarily eaten by populations which have widely different proportions of proteins, fats and carbohydrates in the digestible portions of their food. We are faced with defining the physical properties of fibers from an extraordinarily diverse group of vegetables and fruits, and then trying to study the effects of differing quantities of these materials on the absorption, secretion, digestion and gut flora of humans of differing races and at all the physiologically different stages of their lives. The complexities of this problem have been dealt with in an overview by Eastwood.⁴

Swallowing and Gastric Emptying:

Although it has been postulated⁵ that increasing amounts of dietary fiber will increase the need for mastication and salivation, and thus probably decrease the total food intake, no studies by modern techniques of cineradiography, intraluminal manometry or electrical potentials have yet been carried out on human subjects ingesting different intakes of various dietary fibers. Older studies⁶ showed very little effect of varying the source of hemicelluloses on gastric acidity or emptying.

Gastric Secretion:

Studies now 50 years old provide no evidence that raw or cooked vegetables differ in their ability to provoke gastric secretory activity, all producing responses about 60 percent of those provoked by meat ingestion.⁶ More refined hemicelluloses, like agar-agar, provoke less acid secretion. Studies of the effects of fiber upon pepsin and

intrinsic factor production have not been made.

Small Intestinal Digestion and Absorption:

It is a formidable task to assess the effects on the intraluminal digestion of fats, proteins and carbohydrates exerted by ingesting various levels of the different varieties of dietary fiber. Since one of the features of dietary fibers is their relative insolubility in the fluid phase of the gut, it would probably be necessary to do these complex measurements in isolated loops of animal gut, or by multilumen tube aspiration over a defined segment of small intestine. Bile salts in their conjugated forms do not bind to intraluminal fiber⁷ so it may be assumed that feeding large amounts of fiber will probably not directly affect the micellar phase of fat absorption in the small intestine. There is essentially no available information on the effects of dietary fiber on either digestion or absorption in the small bowel.

Bacterial Flora of the Colon:

It is probable that almost all metabolic functions affected by dietary fiber can be related to the colon. The bacterial flora of the colon can attack and alter those materials which reach it unchanged by the digestive action of the stomach, biliary tree, pancreas and small intestine. There is an enormous literature on this subject dating back over a century; there is little agreement on any phase of it. In addition to the intrinsic difficulties of assessing colonic motility, secretion and bacterial flora, there has been much too glib analogizing between one kind of food fiber to another, without realizing the remarkable differences exerted even by the same fibers when processed in different physical forms. An excellent review is available.⁸

No one currently is satisfied with the methodology for quantitating colonic flora, but on qualitative grounds there is obviously much difference between the relative numbers of anaerobic and aerobic species in the stools of human subjects living in different parts of the world and ingesting different diets. Whether it is the ingestion of certain types of foods or the failure to

ingest other types of foods which make these differences has by no means been established. When one attempts to study this problem by introducing new bacterial species singly into gnotobiotic rats, problems arise. One group of investigators⁹ has concluded that "it is impossible to formulate a general rule about effects of the diet on the number of bacteria in the digestive tract of gnotobiotic rats or on bacterial interaction. Each bacterial species seems to establish individual relations with the host that harbors it, and these relations may or may not be modified by the environment, that is, by the dietary regimen of the host, or by other bacteria harbored." Fiber, as the only variable in human diets, has not been studied in a satisfactory manner.

Although the number of human subjects actually studied is small, the combined investigations in humans and animals reported would show common agreement that over 50 percent of dietary fiber in a western diet can be broken down by bacterial enzymes into short-chain fatty acids, water, carbon dioxide, hydrogen and methane.⁸ If the fiber content is high in lignins, a significantly lower amount is digested by bacteria, and it is possible that lignin-cellulose compounds are totally nondigestible in man.

Fate of Fiber in the Colon

Since so little energy is contributed to the body by dietary fiber, presumably these products must undergo very little colonic reabsorption. Studies by Eastwood¹⁰ demonstrated that the less water-soluble fractions produced will be precipitated, adsorbed to fiber through hydrophobic bonding or absorbed by the bacteria.

Apparently each kind of vegetable fiber studied has different end products, and the fibers which remain undigested have differing affinities for binding the various fractions. An example is the bile acids; deoxycholic acid is largely adsorbed to vegetable fiber in the stool, whereas lithocholic acid is adsorbed to colonic bacteria.

The major anions of normal feces are volatile short-chain fatty acids,¹¹ which at

colonic pH are 99 percent ionized. Being poorly lipid-soluble, they exert an osmotic effect on the colon; they may also provoke colonic peristalsis, although the evidence for this is conflicting. If both actions take place, the bulk and frequency of stools might be increased.

Bacterial action is the sole source of human hydrogen gas production,¹² which can be assayed in the breath. Methane is produced in about one-third of human subjects eating a mixed diet.¹³ It would, therefore, be expected that markedly increasing the quantity of dietary fiber would result in increased flatus, increased breath hydrogen and the passage of more methane in the stools of those who manufacture this gas.

Water Content of Stools

One of the oldest observations about dietary fiber is that its ingestion in increased amounts tends to be associated with larger, wetter stools. Recent studies indicated that polysaccharides can form gels with high water contents, pectic substances are less able to do so, and celluloses least able.¹⁰ The swelling of fibers with water creates networks trapping electrolytes and organic acids in the right colon. Presumably these osmotically active particles in the fiber network create electrical charges which influence further imbibition of water.

The ability to form highly aqueous gels varies enormously among the various fruit and vegetable fibers; the seaweed jelly carrageenan can form gels which are 99 percent water. There is general agreement that the addition of vegetable, fruit or cereal fibers to the standard diet can increase the bulk of the stool, as can the addition of commercially produced powders made from various plant seeds or gums. Wheat bran is a rather dense material and holds less water than does dried vegetable fiber, yet many studies have shown that the addition of 30 to 40 g of dry wheat bran to the daily human diet will increase fecal wet weights by about 60 percent.

A major puzzle for many years has been the failure of materials shown in test tubes

to absorb enormous volumes of water to have the same effect when fed to humans. Physicians have long been disappointed by these failures, and have noted that even in constipated subjects the efficacy of these dietary additions does not necessarily persist. Classical studies of Tainter et al.¹⁴ on the relative water-holding properties of materials used for increasing the bulk of the stools showed poor correlations between the *in vitro* tests and the clinical results. Is it possible that the effect of the fiber is not so much related to its water-holding properties as to some more subtle metabolic effect, possibly the generation of cathartic hydroxylated acids? Current evidence makes this unlikely.¹⁵

Transit Time

A very crude measure of motility is the time required for a labeled meal to be excreted as feces. The literature is full of claims that one or another bulk laxative or food shortens this time when it is desired, especially in the treatment of constipation.

The frequent bowel movements of various native populations eating non-western diets have been applauded and ascribed to high dietary fiber intakes. When careful studies are made of transit times in western subjects, the results are extremely unpredictable. If subjects have what they regard as normal bowel habits, it has proved difficult to influence their transit times by adding fiber to the diet. This suggests, as noted previously, that diets very high in fiber may also be deficient in something else. In constipated western subjects, one can usually demonstrate some increased rapidity of transit, by whatever method, after the subjects have been ingesting 30 g of wheat bran daily for some weeks, or after various placebos.¹⁶ In subjects with regular bowel habits, Findlay failed to change the transit times by bran feeding despite satisfactory increases in stool weight.¹⁷

Possible Relationship with Human Diseases

Since no one can agree on exactly what dietary fiber is or how to measure it, it is impossible to define whether or not fiber

deficiency or excess in the diet exists, or can constitute a protective or noxious element in the diet. Furthermore, to analogize from one kind of dietary fiber—e.g., dried carrots, seaweed, psyllium seed—to another—e.g., wheat bran, corn husks—without controlling the other elements of the diet is to make little contribution to our understanding.

Thus far there are two types of evidence regarding the effects of increasing the fiber content of human diets. One is an epidemiologic observation that some African populations eating large amounts of dietary fiber have less colonic polyposis, inflammatory colitis and enteritis, and cancer of the colon than do western populations. Conversely, one might say that these same populations have more intestinal parasitism because they eat more fiber!

The other type of evidence is pharmacological, and concerns the improvement of symptoms in patients with painful diverticular disease when fed increasing amounts of dietary fiber. Since the great mass of diverticulosis in western populations occurs in older subjects without much history of bowel disturbance, and without muscle hypertrophy in the colon, the group of patients with painful diverticular disease probably represents a segment of the population with irritable colon syndrome, a very common chronic, but usually not very severe, problem beginning in young adult life. To the extent that feeding 15 to 30 g of wheat bran daily can be shown to decrease the increased intrasigmoidal luminal pressures in such patients, these subjects clearly can use more dietary fiber in their diets. There is no evidence that having had less dietary fiber for decades led to the development of the disorder, nor does it explain why all their siblings eating the same diets never develop the disorder.

Atherosclerosis and Appendicitis:

Burkitt postulated that atherosclerosis, particularly coronary and aortic diseases, and appendicitis are as uncommon in native Africans as are the colonic neoplasms discussed below.¹⁸ There is no evidence that such diseases are related to fiber in the diet;

indeed, the annual incidence of acute appendicitis in the United States has shown a distinct downturn in recent decades. The incidence of colon cancer, however, has remained constant in the same population.

Protection Against Toxicants

There is no conclusive evidence that dietary fiber plays an important role in serum cholesterol concentrations in man.⁸ An excellent review of the role of plant fibers in counteracting the toxic effects of drugs, chemicals and food additives has been provided by Ershoff.¹⁹ The protective factors have yet to be identified, and their role in human nutrition is currently unknown.

Neoplastic Disease of the Colon:

On the basis of their rarity in African natives ingesting diets rich in fiber, diverticulosis, polyposis and cancer of the colon in western populations have been attributed by Burkitt to "fiber deficiency" in the diets of the latter. Furthermore, corollaries of the original theory postulated that bacterial flora in western man contains more anaerobes which can degrade neutral or acid sterols, particularly bile acids, cholesterol and their metabolites, into forms which are carcinogenic. The high fiber intake presumably would, by increasing transit, prevent these carcinogens from remaining in contact with the susceptible colonic mucosa long enough to produce neoplastic change.

This interesting theory has stimulated much investigative work, which may be summarized as follows:

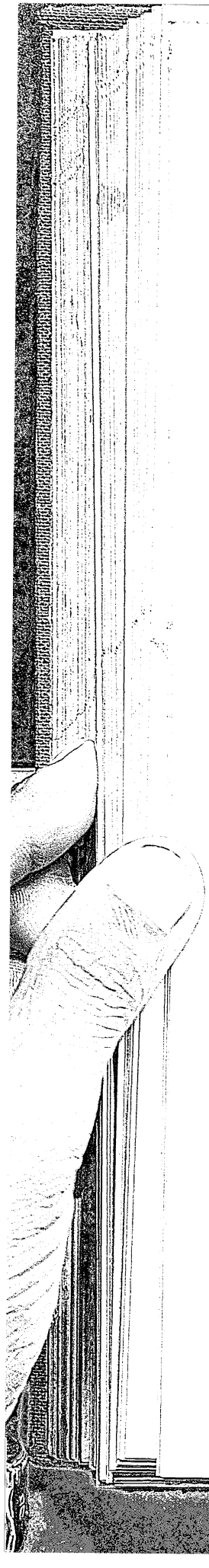
- (1) There is no relationship between transit times in western populations and the incidence of colonic cancer— i.e., constipated subjects do not develop more cancer than non-constipated subjects. Burkitt and others agree that all Westerners need more, larger, softer stools per day to reduce their susceptibility to a gamut of disorders now attributable to "degenerative changes."
- (2) There is no evidence that dietary fiber per se has a definable effect on human bacterial flora.²⁰

- (3) There is very good evidence that anaerobic bacteria flourish when the diet is high in fat and in meat, particularly beef.^{21, 22}
- (4) There is excellent evidence that populations consuming much beef or saturated fats excrete much larger amounts of bile acid metabolites in the stools.²⁰

The synthesis of these observations into a theory of carcinogenesis at this time is speculative. That the diet represents a very important environmental cause for cancer of the colon, the most common serious malignancy affecting both sexes in the United States, is heartening and challenging. There is currently no evidence that the metabolites of neutral or acidic sterols are either carcinogens or cocarcinogens in man, although they resemble some other potent carcinogens in structure, and may be weakly cocarcinogenic in animals.

Although dietary fiber may not be directly protective against human carcinogenesis or other toxic action, this deserves further study. It is certainly consistent with current ideas about energy conservation that we should eat more cereal grains directly, rather than indirectly as beef. We are indebted to Dr. Burkitt and his colleagues for stimulating interest and much research into this very important problem.□

-
1. P.J. Van Soest and R.W. McQueen, *Proc. Nutrition Soc.* 32: 123-130, 1973
 2. D.A.T. Southgate, *Proc. Nutrition Soc.* 32: 131-136, 1973
 3. D.A.T. Southgate and J.G.V.A. Durnin, *Brit. J. Nutrition* 24: 517-535, 1970
 4. M.A. Eastwood, N. Fisher, C.T. Greenwood and J.B. Hutchinson, *Lancet* I: 1029-1032, 1974
 5. K.W. Heaton, *Lancet* II: 1418-1421, 1973
 6. P.B. Hawk, M.E. Rehfuess and O. Bergeim, *Am. J. Med. Sci.* 171: 359-369, 1926
 7. M. A. Eastwood and D. Hamilton, *Biochim. Biophys. Acta* 152: 165-173, 1968
 8. J.H. Cummings, *Gut* 14: 69-81, 1973
 9. P. Raibaud, R. Ducluzeau, M-C. Muller and G.D. Abrams, *Am. J. Clin. Nutrition* 25: 1467-1474, 1972

- 
10. M.A. Eastwood, *Proc. Nutrition Soc.* 32: 137-143, 1973
 11. J.S. Fordtran, *New Engl. J. Med.* 284: 329-330, 1971
 12. M.D. Levitt, *New Engl. J. Med.* 281: 122-127, 1969
 13. M.D. Levitt and J.H. Bond, Jr., *Gastroenterology* 59: 921-929, 1971
 14. M.L. Tainter and O.H. Buchanan, *Ann. N.Y. Acad. Sci.* 58: 438-452, 1954
 15. H.S. Wiggins, J.R. Pearson, J.G. Walker, R.I. Russell and T.D. Kellock, *Gut* 15: 614-621, 1974
 16. T. Greiner, I. Bross and H. Gold, *J. Chron. Dis.* 6: 244-255, 1957
 17. J.M. Findlay, W.D. Mitchell, A.N. Smith, A.J.B. Anderson and M.A. Eastwood, *Lancet* i: 146-149, 1974
 18. D.P. Burkitt, A.R.P. Walker and N.S. Painter, *J. Am. Med. Assn.* 229: 1068-1074, 1974
 19. B.H. Ershoff, *Am. J. Clin. Nutrition* 27: 1395-1398, 1974
 20. M.J. Hill, *Am. J. Clin. Nutrition* 27: 1475-1480, 1974
 21. E.L. Wyndner and B.S. Reddy, *Cancer* 34: 801-806, 1974
 22. S.M. Finegold, H.R. Attebery and V.L. Sutter, *Am. J. Clin. Nutrition* 27: 1456-1469, 1974

Nutrition Reviews

authoritative articles by distinguished international scientists • competent, critical, and knowledgeable reviews • interpretative summaries of important original clinical and experimental research • selected excerpts from classic publications marking milestones in nutrition research • informative special reports on government and organization actions • contributing editors review journals of widespread interest covering biochemistry, food science, public health, toxicology, dentistry, pediatrics, internal medicine, gastroenterology and other clinical specialties •

Send subscription and renewal requests to:

THE NUTRITION FOUNDATION
888 Seventeenth Street, N.W.
Washington, D. C. 20006

\$12.00 per year

\$6.00 for students

ACRODERMATITIS ENTEROPATHICA--HEREDITARY ZINC DEFICIENCY

An inherited defect in zinc absorption is apparently the cause of acrodermatitis enteropathica. Complete remission of symptoms is obtained with doses of zinc sulfate above the RDA.

Key Words: acrodermatitis enteropathica, zinc, diiodohydroxyquin, zinc deficiency

"Acrodermatitis enteropathica (AE) is a hereditary disease appearing in early infancy, characterized by acral and orificial vesicobullous, pustular and eczematoïd skin lesions, alopecia and diarrhea. The inheritance pattern is believed to be autosomal recessive with nearly equal incidence in male and female patients. The onset of symptoms usually occurs within the first few months of life and is frequently associated with the change from breast feeding to cow's milk. In its full genetic expression, the usual course without treatment is a relentless progression of severe malnutrition, poor growth and development, intercurrent bacterial and yeast infections, general debility and death within one to three years."¹ Many other types of complications may occur and there is apparently some degree of phenotypic heterogeneity since some cases with milder manifestations, delayed onset and simultaneous resolution have been reported.

Since 1953 the disease has been treated empirically with oral diiodohydroxyquin (Diodoquin) with rapid and dramatic resolution of the disease. Apparently most patients require treatment indefinitely. Since treatments were first started in 1953, about 20 years is the longest follow-up period to date.

That the diet was somehow involved in this disease was indicated by the usual development when breast feeding was terminated. Neldner et al.¹ reported rather extensive studies on one adult who developed the disease at six weeks of age

when breast feeding was stopped. Several simplified diets were tried. Tryptophan metabolism with and without pyridoxine supplements, glucose tolerance and insulin levels, and serum lipoprotein levels were studied as well as the light and electron microscopic patterns of the jejunal mucosa. The kinds of studies performed reflect the reasonable assumption that there might be an absorptive defect or that abnormal metabolism of some of the nutrients in which dermatitis is a deficiency symptom—tryptophan, pyridoxine, essential fatty acids—might be involved. However, the last paragraph of this paper calls attention to a Letter to the Editor of Lancet from Moynahan and Barnes² in which an infant with acrodermatitis enteropathica and an associated lactose intolerance was treated with zinc sulphate. This patient improved somewhat on a lactose-free diet but this was not maintained. A careful calculation of the micronutrient content of the diet suggested zinc deficiency and there was a complete recovery of her skin lesions and bowel symptoms within a short period of time after zinc administration. Neldner et al. reported that subsequent to the submission of their paper, their patient was given zinc sulfate, the diiodohydroxyquin was discontinued and there was a prompt remission which had been maintained for six weeks at the time the report was made.

Confirmation of the role of zinc has come rapidly. Moynahan followed his report with another Letter to the Editor of Lancet³ with the title, "Acrodermatitis Enteropathica: A Lethal Inherited Human Zinc Deficiency Disorder." He reported

that nine patients given zinc were completely free of symptoms. Thirty-five mg of zinc sulfate daily appeared to "suffice to bring about and maintain complete relief of symptoms. Nevertheless, the optimum intake seems to be 150 mg of zinc sulphate in divided doses, in order to deal with intestinal infections and the like, in which diarrhea may reduce availability of the element. Pubescent children, especially boys, may require increased amounts through adolescence. . ." Moynahan points out that on the basis of previous ultrastructural and enzyme histological studies^{4,5} and the prompt relief that followed when breast milk was fed, that they suggested that there was an absence or defect in an oligopeptidase. He postulated that the noxious factor was a peptide which was produced from most proteins but not from breast milk protein and this peptide was not hydrolyzed in these patients. In view of the response to zinc he suggested that the noxious peptide chelates zinc and reduces the availability of dietary zinc to the patient. Presumably diiodohydroxyquin would exert its favorable effect by chelating zinc and assisting in its absorption.

Neldner and Hambidge⁶ reported more fully on the use of zinc in their patient, a 22-year-old woman, who had the first signs of the disorder at three months of age and was near death at one and one-half years of age when diiodohydroxyquin treatment was first started. She responded rapidly and continued to require the drug, 650 mg, three or four times daily. Plasma zinc in this patient was of the order of 40 μg per 100 ml while on her usual diet and receiving diiodohydroxyquin. Treatment with zinc sulfate, 220 mg three times daily, resulted in an increase in the plasma zinc to approximately 110 μg per 100 ml. All therapy was discontinued and her disease began to reoccur after five weeks. The plasma zinc was then extremely low, <20 μg per 100 ml. Diiodohydroxyquin therapy was begun and the patient gradually responded. The plasma zinc level rose to approximately 30 μg per 100 ml. When the drug

was discontinued "there was a rapid clinical deterioration over the next four days and the plasma zinc concentration fell to 10 μg per 100 ml."

A "low dosage" zinc therapy was started (50 mg zinc sulfate, twice daily). There was a clinical response within 24 hours and remission was complete within five days. The plasma zinc level began to rise and was 70 μg per 100 ml two weeks later and still rising. Serum alkaline phosphatase levels, which had previously been well below 20 U per liter, rose to normal levels. Urinary zinc excretion which was approximately 50 μg per 24 hours during diiodohydroxyquin treatment rose rapidly to normal levels after zinc administration. Normal zinc excretion is considered to be between 174 to 888 μg per 24 hours.

Confirmation of the efficacy of zinc administration has also been reported by Michaelsson⁷ and by Thyresson.⁸

It appears, therefore, that this inherited disorder is explained as a severe zinc deficiency. The various authors noted some of the symptoms of zinc deficiency in animals which may be comparable—growth retardation, impaired keratogenesis, including alopecia and skin lesions, lethargy and behavioral changes, increased susceptibility to infections and possibly impaired development of the immune system. Although hypogeusia (impairment of taste acuity) has been reported in zinc deficiency, the patient studied by Neldner did not show this abnormality even when off therapy.

The defect in these patients apparently must be in the absorption of zinc although the mechanism is as yet unclear. Lombeck et al.⁹ studied the absorption of ⁶⁵Zn in three patients. They reported values of 15, 30 and 42 percent compared to 58 to 77 percent in control subjects.

In commenting editorially in *The Lancet*¹⁰ on these studies the editors conclude that, "Moynahan's discovery has implications far beyond the small group of seriously ill patients with acrodermatitis enteropathica who will be the immediate beneficiaries; it should be a powerful impulse to

all interested in zinc metabolism to look for other manifestations of deficiency, either natural or induced, in both children and adults." □

1. K. H. Neldner, L. Hagler, W. R. Wise, F. B. Stifel, E. G. Lufkin and R. H. Herman: Acrodermatitis Enteropathica. Clinical and Biochemical Survey. *Arch. Dermatol.* 110: 711-721, 1974
2. E. J. Moynahan and P. M. Barnes: Zinc Deficiency and A Synthetic Diet for Lactose Intolerance. *Lancet* I: 676-677, 1973
3. E. J. Moynahan: Acrodermatitis Enteropathica: A Lethal Inherited Human Zinc-Deficiency Disorder. *Lancet* II: 399-400, 1974
4. E. J. Moynahan: Acrodermatitis Enteropathica with Secondary Lactose Intolerance, and Tertiary Deficiency State due to Chelation of Essential Nutrients by Di-iodohydroxyquinolone. *Proc. Roy. Soc. Med.* 59: 445-447, 1966
5. E. J. Moynahan, F. R. Johnson and R. M. H. McMin: Acrodermatitis Enteropathica: Demonstration of Possible Intestinal Enzyme Defect. *Proc. Roy. Soc. Med.* 56: 300-301, 1963
6. K. H. Neldner and K. M. Hambidge: Zinc Therapy of Acrodermatitis Enteropathica. *New Engl. J. Med.* 292: 879-882, 1975
7. G. Michaelsson: Zinc Therapy in Acrodermatitis Enteropathica. *Acta Dermatol.* (Stockholm) 54: 377-381, 1974
8. N. Thyresson: Acrodermatitis Enteropathica. Report of a Case Healed with Zinc Therapy. *Acta Dermatol.* (Stockholm) 54: 383-385, 1974
9. I. Lombeck, H. G. Schnippering, F. Ritzl, L. E. Feinendegen and H. J. Bremer: Absorption of Zinc in Acrodermatitis Enteropathica. *Lancet* I: 855, 1975
10. Zinc in Human Medicine. *Lancet* II: 351-352, 1975

ESSENTIAL FATTY ACID DEFICIENCY IN CONTINUOUS-DRIP ALIMENTATION

Essential fatty acid deficiency as judged by altered serum lipid levels of linoleic and eicosatrienoic acids was produced quickly in adult humans by constant infusion of a fat free nutrient solution.

Key Words: essential fatty acids, linoleic acid, intravenous feeding, humans

Essential fatty acid (EFA) deficiency occurs in animals receiving diets low in both linoleic (18:2 ω 6) and arachidonic (20:4 ω 6) acids. The deficiency symptoms may be completely prevented by the inclusion of either of these acids in the diet at a level approximating 1 percent of the calories.¹ Although arachidonic acid is the physiologically essential acid, its ease of synthesis in vivo from linoleic acid and the wide distribution of the latter in food lipids makes linoleic acid the most practical dietary source of EFA.²

Studies of human EFA deficiency have been almost exclusively made with infants and children³ and showed this deficiency to be preventable by dietary linoleate. A better understanding of the role of EFA in adults is important in view of the wide-

spread use of long term intravenous alimentation employing lipid free infusates. Wene, Connor and DenBesten⁴ reported that changes in serum fatty acid profiles occurring in healthy men fed fat free diets quickly change to resemble those of EFA-deficient animals and human infants.

In the first of two studies a hyperalimentation diet was given intravenously and nasogastrically to eight healthy adult male volunteers. This fat free diet contained 80 percent glucose and 20 percent casein amino acid hydrolysate. Appropriate electrolytes and water-soluble vitamins were added. Sterile solutions were prepared daily to provide 1 kcal per milliliter. Individual caloric requirements were assessed on the basis of dietary history and height-weight measurements. A seven day preliminary period on a general diet containing linoleic acid at 2 to 3 percent of calories was fol-

lowed by two sequential 14 day periods on the fat free diet. Four subjects first received the diet intravenously and then nasogastrically. The treatment route sequence was reversed for the other four. In all cases the solutions were infused at a constant rate over 24 hours. The subjects were ambulatory and engaged in usual ward recreational activities.

In the second study a single healthy adult male was first fed a formula diet containing 2.6 percent of calories as linoleic acid for two months. This was followed by a completely fat free diet administered by constant rate nasogastric drip for ten days, then 24 hours of fast. Then the same diet was given for three days intermittently by nasogastric tube at 8 AM, 12 noon and 4 PM. A 14 day repletion period followed during which the initial formula diet containing linoleic acid was fed.

Serum lipids were separated by thin layer chromatography. The fatty acid composition of the separated lipid classes was determined by gas-liquid chromatography of methyl esters of the fatty acids. In study one, data are presented for one sample per subject at the end of the basal and the two experimental periods. Samples were taken more frequently from the single subject of study two. Data are presented as percent of total fatty acids.

In study one all men initially had normal distribution of serum fatty acids. Biochemical evidence of EFA deficiency developed within two weeks in all subjects when given the fat free regimen. Linoleic acid levels fell and eicosatrienoic acid levels rose in all serum lipid fractions. The largest percent change for linoleic acid, from 21.2 to 3.2 percent, was in the phospholipid fraction. Eicosatrienoic acid was undetectable in normal serum lipid fractions and rose to a maximum of 2.5 percent in the phospholipid fraction. Arachidonic acid levels did not change consistently in any lipid fraction. The second two weeks of fat free feeding did not materially alter the results, nor did reversal of the order of routes of administration. On a percent basis the loss of linoleic acid was compensated

by a gain of palmitoleic and oleic acids. Dermatitis or other signs of EFA deficiency were not seen.

In the second study linoleic acid decreased in all serum lipid fractions by the third day of the initial fat free feeding period and continued to decrease to day nine of the ten day period, finally reaching 10 to 20 percent of the control period values. Eicosatrienoic acid was not measurable in the control period serum but appeared on the first day of fat free feeding in the triglyceride and phospholipid fractions. It continued to increase in these fractions during the ten day period to a maximum of 2.9 percent. Arachidonic acid did not change.

During the 24-hour fast linoleic acid rose moderately in all fractions, most obviously in the free fatty acid fraction.

During the three day intermittent feeding period on the fat free diet linoleic acid levels doubled in the cholesterol ester fraction but did not change remarkably in the other fractions. Eicosatrienoic acid did not change during the 24-hour fast and decreased markedly during the intermittent feeding period only in the triglyceride fraction. During the repletion period linoleic and eicosatrienoic acid levels changed steadily and reached baseline levels by day 13. No clinical signs of EFA deficiency were seen in this subject.

These studies clearly indicate that biochemical changes indicative of EFA deficiency can be detected in adult humans continuously fed fat free infusions high in glucose. It is also suggested, on the basis of a single individual, that intermittent feeding of this infusate permits a partial repletion of EFA. The authors suggest, logically, that this repletion may be a result of lipolysis during the periods that the tissues are not flooded with glucose. This would release linoleate from lipid depots which contain about 12 percent of this fatty acid. Continuous infusion, on the other hand, would be expected to reduce lipolysis and thus render linoleate stores unavailable to maintain serum levels. While this concept is of theoretical interest, the practical solu-

tion to the problem would be inclusion of linoleate in infusion mixtures. If nutrition by infusion is to be maintained for extended periods it would also seem appropriate to include sources of the fat-soluble vitamins, not used in the experiments reported here. □

1. R. T. Holman: Essential Fatty Acids. *Nutrition Reviews* 16: 33-35, 1958
2. J. J. Rahm and R. T. Holman in *The Vitamins, Chemistry, Pathology, Methods*. W. H.

Sebrell and R. S. Harris, Editors, Vol. III, p. 601. Academic Press, New York, 1971

3. H. F. Wiese, A. E. Hansen and D.J.D. Adam: Essential Fatty Acids in Infant Nutrition. I. Linoleic Acid Requirement in Terms of Di-, Tri- and Tetraenoic Acid Levels. *J. Nutrition* 66:345-360 1958
4. J. D. Wene, W. E. Connor and L. DenBesten: The Development of Essential Fatty Acid Deficiency in Healthy Men Fed Fat-Free Diets Intravenously and Orally. *J. Clin. Invest.* 56: 127-134, 1975

NEW DIAGNOSTIC TESTS FOR VIRAL HEPATITIS A

Several useful methods for detecting hepatitis A are now available.

Key Words: hepatitis A, viral hepatitis

Viral hepatitis as seen in Europe and North America can be caused by many different diseases of which epidemic hepatitis, short incubation period hepatitis or virus A is the most common; serum hepatitis or virus B the most serious; and mononucleosis, measles and a variety of enteric viruses occasionally produce identical clinical illness. The clear discovery of serological methods for detection of hepatitis B (HB) has made the clinical, transmission, and epidemiological features of this disease much clearer. Hepatitis A has, however, resisted such testing until quite recently when three separate methods appeared, all of which seem well founded and have been confirmed.

Hilleman and co-workers^{1,2} used the marmoset model to develop an antigen. Liver extracts prepared from marmosets infected with hepatitis A were employed in two separate assays. The specificity of the marmoset isolation of human hepatitis A for the CR 326 strain was confirmed by this same group^{3,4} laying the groundwork for assay techniques.

The critical evaluation of both tests came from utilizing the huge bank of frozen sera from all types of hepatitis preserved by Krugman and co-workers.⁵ Their group undertook both tests in Hilleman's laboratory, with blind coded sera

and the results were highly specific for both tests. A complement fixation test was developed in which control acute serum had antibody titers of 1:160 to 1:1280 and those without disease had levels always less than 1:10. This test was highly specific for acute hepatitis A and levels of antibody remained high for up to ten years. Other conditions did not give positive results.

An immune adherence hemagglutination test was also developed. Antigen-antibody complement complexes adhere to human erythrocytes and this is the basis of a sensitive assay. In this assay control patients never exceeded titers of 1:5 but acute phase and chronic titers always exceed 1:640. This test was the most sensitive and was abnormal in 45 percent of persons in the first week of illness and in all by the fourth week of illness.

A group at the National Institutes of Health, working on virus identification in the stool by electron microscopy of stool specimens, applied this to the hepatitis A problem. Techniques for the examination of whole stool specimens are now well refined and virus particles can be identified. This is rendered specific by studying the samples before and after treating with specific antisera for the antiserum causes agglutination of the virus with which it combines. In hepatitis A Feinstone and

collaborators⁶ found a small cubic virus with a diameter of 27 mm. This has been confirmed by the Hilleman group.⁷

It is now apparent that methods for identifying hepatitis A are with us and should be widely available on a research basis within the next few years. This should clarify the epidemiology and lead to an effective vaccine in the next few years. □

1. P. J. Provost, O. L. Ittensohn, V. M. Villarejos and M. R. Hilleman: A Specific Complement-Fixation Test for Human Hepatitis A Employing CR 326 Virus Antigen: Diagnosis and Epidemiology. *Proc. Soc. Exp. Biol. Med.* 148: 961-968, 1975
2. W. J. Miller, P. J. Provost, W. J. McAleer, O. L. Ittensohn, V. M. Villarejos and M. R. Hilleman: Specific Immune Adherence Assay for Human Hepatitis A Antibody. Application to Diagnostic and Epidemiologic Investigations. *Proc. Soc. Exp. Biol. Med.* 149: 254-261, 1975
3. C. C. Mascoli, O. L. Ittensohn, V. M. Villarejos, J. A. Arguedas G., P. J. Provost and M. R.

Hilleman: Recovery of Hepatitis Agents in the Marmoset from Human Cases Occurring in Costa Rica. *Proc. Soc. Exp. Biol. Med.* 142: 276-282, 1973

4. P. J. Provost, O. L. Ittensohn, V. M. Villarejos, J. A. Arguedas G., and M. R. Hilleman: Etiologic Relationship of Marmoset-Propagated CR 326 Hepatitis A Virus to Hepatitis in Man. *Proc. Soc. Exp. Biol. Med.* 142: 1257-1267, 1973
5. S. Krugman, H. Friedman and C. Lattimer: Viral Hepatitis, Type A. Identification by Specific Complement Fixation and Immune Adherence Tests. *New Engl. J. Med.* 292: 1141-1143, 1975
6. S. M. Feinstone, Z. A. Kapikian and R. H. Purcell: Hepatitis A: Detection by Immune Electron Microscopy of a Viruslike Antigen Associated with Acute Illness. *Science* 182: 1026-1028, 1973
7. P. J. Provost, B. S. Wolanski, W. J. Miller, O. L. Ittensohn, W. J. McAleer and M. R. Hilleman: Physical, Chemical and Morphologic Dimensions of Human Hepatitis A Virus Strain CR 326. *Proc. Soc. Exp. Biol. Med.* 148: 532-539, 1975

IRON SUPPLEMENTATION FOR GESTATIONAL ANEMIA: A MODEL FIELD TRIAL

An elegantly designed study showed only a modest impact on gestational anemia even at high levels of supplemental iron, folic acid and vitamin B₁₂.

Key Words: hemoglobin, hematocrit, supplemental iron, anemia, pregnancy

Iron deficiency, as manifested by sub-optimal to frankly anemic hematologic values, is a well documented health problem of vast scope. It is endemic in developed as well as in developing areas of the globe. In terms of severity within population groups, pregnant women are especially vulnerable owing to the extra demands of fetal, placental and decidual tissues for iron as well as for the 10 to 15 percent expansion of maternal red blood cell mass accompanying pregnancy.

A hemoglobin of less than 11 g per 100 ml has been used as a criterion of anemia in pregnancy. By this standard, some 80 percent of pregnant women surveyed in India are anemic, almost entirely owing to iron

deficiency. For this reason the World Health Organization sponsored a collaborative field trial within India to assess the impact of iron supplementation upon gestational anemia and upon the outcome of pregnancy. The report of that trial¹ is the basis for this review.

Women were identified and screened at 22 ± 2 weeks of gestation in two geographically separate areas of India. Those not excluded for reasons of chronic diseases, chronic diarrhea or because of a hemoglobin of less than 5 g per 100 ml were randomized into two preliminary treatment groups. One group was given oral folic acid, 5 mg daily and parenteral vitamin B₁₂, 100 µg biweekly. The other group received placebo tablets and injections on the same schedule. For the four weeks of this phase,

hemoglobin values fell slightly and equally in both groups so that at the onset of the definitive phase of the study there were no differences in hemoglobin (overall mean 9.5 g per 100 ml with 87 percent having a level of less than 11 g per 100 ml) or in hematocrit (overall mean of 32.2 percent) between treatments or between geographic areas.

For the final trial, the women were randomized into seven groups. The preliminary folate-B₁₂ placebo group was divided in half: group 0 continuing on the identical placebo regimen; group 6 receiving 120 mg of daily iron with no folic acid and with placebo injections. The preliminary group treated with folate-B₁₂ was split into five groups (one through five), all of which were continued on the two vitamins at the same dosage schedule and varying only in the amount of daily iron: 0, 30, 60, 120 and 240 mg in groups one through five respectively. All tablets (iron only, iron plus folate or placebo) looked alike, were taken as a divided daily dose six days per week and were consumed under the direct observation of a public health nurse. In all these groups, the form of the iron was ferrous fumarate. This phase lasted ten to 12 weeks. The study terminated at 36 to 38 weeks of gestation. Potency of the several iron levels was checked by chemical analysis, and efficacy assessed by administration to four grossly iron-deficient nonpregnant females having initial hemoglobin levels of about 4 g per 100 ml. Each showed a persistent and substantial hematologic response to the 30 and 60 mg daily iron supplements over an 80 day test period.

Some 647 women completed the definitive study, group sizes ranged from 70 to 115. Presentation of results was largely based on paired statistical analyses of the initial and final venous blood values of these 647 individuals. The mean of paired individual changes in groups 0 and 1 (no iron) were, respectively, -0.37 and -0.22 g per 100 ml for hemoglobin and -0.4 and 0.0 percent for hematocrit. On the other hand, groups two through five showed

significant dose-related responses in all hematologic parameters. The magnitude of the change, however, was small; a 1.4 g per 100 ml increase in hemoglobin and a 3.8 percent increase in hematocrit in group five (240 mg daily iron) being the largest, still leaving 59 percent of women in this group with hemoglobins under 11 g per 100 ml at the end of the study. Hemoglobin response in group six (120 mg iron only) was about half the response in group four (120 mg iron plus folate-B₁₂), +0.7 versus +1.3 g per 100 ml respectively. This suggests a role for these vitamins in the gestational anemia of this population group.

There was no relationship between hemoglobin response and serum albumin (taken as an index of protein nutriture) nor was there a demonstrable relationship between hookworm infestation (41 percent prevalence initially) and either initial or final hematologic assessment. Data on serum iron and percent transferrin saturation were also presented. While amply confirming the presence of iron deficiency, responses of these parameters generally mirrored the hemoglobin-hematocrit responses.

Birth weights were available in 301 infants; there were no differences among the various experimental groups. Three month postpartum hemoglobin levels were presented for 243 mothers and 269 infants. For the former, the only residual difference was a slightly higher hemoglobin in the group five mothers (0.6 g per 100 ml higher, $p < .05$). There were no differences among the 269 infants.

Why such small hematologic responses? The authors have made some calculations of supplemental iron absorption in these groups. Assuming that the fetal and placental iron stores were constant (there were no group differences in three month infant hemoglobins) and thus assuming that all absorbed iron was utilized for maternal hemoglobin production, percent absorption of iron was estimated. These estimates are extraordinarily low, ranging from 6.7 percent in group two to 1.4 percent in group five. These are lower than cited literature

values for nonpregnant anemic subjects and are one-quarter the calculated absorption in the four nonpregnant subjects reported in this study. Neither folate nor B_{12} was limiting in groups one through five. Were there physical or dietary conditions which limited iron availability, absorption or hemoglobin synthesis in these village women? While the answers are not known, reports of disappointing hematologic responses to supplemental iron in gestational anemia in other countries are cited by the authors.

From a public health point of view, the persistence of a high frequency of anemia in spite of supplementation programs raises the traditional Procrustean dilemma in allocation of limited resources: whether to identify and treat the most severely anemic pregnant women (a logistically complex and expensive intervention mode) or whether to supplement the whole population at risk even though oral hematinic therapy is demonstrably incapable of reversing or even significantly mitigating the condition?

This also touches upon a further problem: does "borderline" anemia, in contrast to "severe" anemia, really impair health in any demonstrable way? If so, it has so far eluded detection and quantification. In an earlier review² the failure to relate various middle-aged health-related symptoms to hematologic values was reviewed. More

recently, Elwood³ was unable to associate hematocrit, in the borderline area, with mortality in 18,740 women. The classic Vanderbilt study⁴ failed to show a relationship between maternal hemoglobin level and outcome variables such as birth weight, infant hemoglobin or risk of anemia in the first year of life. This comparison involved the lowest and highest hemoglobin quartiles, group mean hemoglobins being 9.9 and 13.1 g per 100 ml respectively.

Thus the functional impact of gestational anemia is not clear, nor has the health impairment associated with sub-optimal iron nutriture among adults been clearly demonstrated. □

1. S. K. Sood, K. Ramachandran, M. Mathur, K. Gupta, V. Ramalingaswamy, C. Swarnabai, J. Ponniah, V. I. Mathan and S. J. Baker: W.H.O. Sponsored Collaborative Studies on Nutritional Anaemia in India. I. The Effects of Supplemental Oral Iron Administration to Pregnant Women. *Quart. J. Med.* 44: 241-258, 1975
2. Symptoms of Iron Deficiency Anemia. *Nutrition Reviews* 25: 86-87, 1967
3. P. C. Elwood, W. E. Waters, I. T. Benjamin and P. M. Sweetnam: Mortality and Anaemia in Women. *Lancet* i: 891-894, 1974
4. C. W. Woodruff and E. B. Bridgeforth: Relationship Between the Hemogram of the Infant and That of the Mother During Pregnancy. *Pediatrics* 12: 681-685, 1953

IMMUNE DEFICIENCY IN MALNUTRITION

Once again it is shown that a defect in cellular immunity exists in malnutrition. The clinical significance of such a finding is that malnourished children may be more prone to fungal and gram-negative infections.

Key Words: immune competence, malnourished, immunoglobins, cellular immunity, immunodeficiency

There are now several studies which document the relationship between malnutrition and infection.^{1,2} Scrimshaw and his colleagues, who perhaps have done the most to promote this association, commented in detail on the vicious cycle which may be set in train when malnutrition predisposes

to infection which in turn produces a greater degree of malnutrition.^{3,4} The major reason for newer studies would be to use newer tests of immune competence or to identify various aspects of the malnutrition process which may be more specifically related to immunodeficiency. The study of Smythe et al., which has already been reviewed,⁵ presented data partly based on autopsy material showing that

there was a defect in cellular immunity. More recent work showed that there may be a specific T cell abnormality.⁶

A recent study on Ghanaian children presents further detailed evidence of a defect in cell-mediated immunity.⁷ In addition an attempt was made to find meaningful correlations between various indices of malnutrition and measures of immune competence. One hundred and seventeen children were divided into three groups. There was a group of controls which consisted of children above 81 percent of the Harvard standard, had normal serum protein levels and were normal at clinical examination. The malnourished children were divided into two groups on the basis of clinical examination, anthropometric and laboratory data. The group of severely malnourished children could be divided into those with marasmus and those with kwashiorkor, but all were less than 60 percent of the Harvard standard. The group with moderate malnutrition was 61 to 80 percent of the Harvard standard and came from nutritional rehabilitation units.

Clinically obvious infections were most common in the severely malnourished children; 18 percent had skin fungus and 15 percent had pneumonia. Pyoderma occurred in all groups of children. It was striking to note that 3 percent of the severely malnourished children had tuberculosis. Infestation with *Strongyloides stercoralis* was ten times more common in the severely malnourished compared with other children, but ascariasis and giardiasis were equally frequent in all groups. Clinical assessment of tonsil size showed that in 36 percent of the malnourished children the tonsils were not visible or barely visible and in general, the better nourished the children, the larger the tonsils. As perhaps could be predicted, plasma proteins were lowest in the severely malnourished children. The depression of serum albumin and transferrin was most marked in children with kwashiorkor.

Levels of the third component of complement were significantly reduced in the

severely malnourished group and this was also most marked in children with kwashiorkor. It took only two weeks of nutritional rehabilitation for C_3 to increase to normal levels. The levels of all five immunoglobulins were elevated in all the Ghanaian children when the values were compared with those from American children. A comparison within the three groups of Ghanaian children showed that the immunoglobulins were highest in the most severely malnourished children. An interesting observation was that IgE was most markedly elevated in children with intestinal parasites. Malnutrition did not affect the antibody response to keyhole limpet hemocyanin and polyvalent pneumococcal polysaccharide.

The most impressive finding in this study was the depression of the cellular immune response. The mean absolute lymphocyte count and cutaneous delayed hypersensitivity to phytohaemagglutinin, monilia streptokinase-streptodornase were significantly reduced in severely malnourished children. The *in vitro* lymphocyte reactivity to phytohaemagglutinin was significantly reduced in both groups of malnourished children with the severely malnourished ones showing the greatest reduction.

The authors give detailed statistical correlations between several of the things they measured. Tonsil size and the indices of cellular immunity showed correlations with total serum proteins, albumin, carotene, hemoglobin and vitamin C levels. The anthropometric measurements such as weight for age, arm circumference and triceps skin fold showed impressive highly significant correlations with the indices of cellular immunity but also with the serum transferrin; arm circumference and skin-fold thickness were correlated with the serum complement.

This study confirms previous ones which showed depression of cellular immunity in malnutrition.^{1,2,6} In addition, it shows that the extent of the depression is related to the severity of the malnutrition. The authors do point out that malnutrition is

associated with multiple deficiency states and it is impossible to single out one as being primarily responsible for any immunodeficiency. Iron deficiency for example which is a common finding in malnutrition is often associated with cellular immunodeficiency.⁸ The question must now be asked, what is the effect in clinical terms of this immunodeficiency? It is constantly stated and the authors repeat it, that these immunodeficiencies make the malnourished child more prone to infections with gram-negative organisms. The authors also imply that this finding of a low C_3 may be of significance in this regard.

It should be pointed out that it requires much more evidence beside a depression of C_3 in the presence of a normal C_4 to allow any opinion to be given on the functional state of the complement system as a whole or of any of the component parts. Unfortunately the evidence for a greater prevalence of gram-negative infections in malnutrition is represented by a few limited studies.^{9,10} From the authors' own data pneumonia was the most common infection and there is no evidence that pneumonia with gram-negative organisms is more frequent in malnutrition.

The evidence that malnutrition and infection go hand in hand is indeed strong, but perhaps the time has come for attention to be paid to the ecological aspects of the two. The association is not surprising when children who are malnourished often live in the kinds of unsanitary and overcrowded conditions which are the natural breeding grounds of infection. Tuberculosis is common in malnutrition, but the evidence is strong that this infection thrives among people in poor living conditions. Pneumonia is common in malnourished children, but is it the result of a basic immune deficiency or does poor hygiene plus the general weakness and inability to cough properly help in making the child prone to all respiratory infections? The authors point out that skin fungus infection was most common in the severely malnourished child, but this could also be

attributed to a lack of basic sanitation rather than to an immunodeficiency. The mucocutaneous candidiasis which can be seen in cellular immune deficiency states has not been recorded in protein-energy malnutrition.

It is surprising that in a study such as the present one no mention was made of the work in Uganda¹¹ which showed the relationship of infection to malnutrition most elegantly. Episodes of infections occurred while children were relatively well nourished, but because of the convalescent anorexia coupled with the relative unavailability of excess food these children could not exhibit the normal catch-up growth which follows any period of growth failure. These children because of repeated infections showed progressive falls in serum albumin and slow development of clinically obvious kwashiorkor.

From the practical clinical aspect of the treatment of malnourished children, the most important point is not simply the frequency of infections with which the child presents, but the fact that it is very difficult to diagnose these infections. Leukocytosis and pyrexia may be absent, Mantoux tests may be invalid and a high index of suspicion must always be maintained. □

1. R. K. Chandra: Immunocompetence in Under-nutrition. *J. Pediat.* 81: 1194-1200, 1972
2. P. M. Smythe, M. Schonland, G. G. Brereton-Stiles, H. M. Coovadia, H. J. Grace, W. E. K. Loening, A. Mafoyané, M. A. Parent and G. H. Vos: Thymolymphatic Deficiency and Depression of Cell-Mediated Immunity in Protein-Calorie Malnutrition. *Lancet* II: 939-944, 1971
3. N. S. Scrimshaw, C. E. Taylor and J. E. Gordon: Interactions of Nutrition and Infection. World Health Organization, Monograph Series No. 57, 1968
4. J. E. Gordon and N. S. Scrimshaw: Infectious Disease in the Malnourished. *Med. Clin. N. Am.* 54: 1495-1508, 1970
5. Cellular Immunity and Malnutrition. *Nutrition Reviews* 30: 253-255, 1972

6. R. K. Chandra: Rosette Forming T Lymphocytes and Cell-Mediated Immunity in Malnutrition. *Brit. Med. J.* 3: 608-609, 1974
7. C. G. Neumann, G. J. Lawlor, Jr., E. R. Stiehm, M. E. Swenseid, C. Newton, J. Herbert, A. J. Ammann and M. Jacob: Immunologic Responses in Malnourished Children. *Am. J. Clin. Nutrition* 28: 89-104, 1975
8. The Relationship between Infection and the Iron Status of an Individual. *Nutrition Reviews* 33: 103-105, 1975
9. P. M. Smythe and J. A. H. S. Campbell: The Significance of the Bacteraemia of Kwashiorkor. *S. Afr. Med. J.* 33: 777-779, 1959
10. I. Phillips and B. Wharton: Acute Bacterial Infection in Kwashiorkor and Marasmus. *Brit. Med. J.* 1: 407-409, 1968
11. J. D. L. Frood, R. G. Whitehead and W. A. Coward: Relationship between Pattern of Infection and Development of Hypoalbuminaemia Hypo β -Lipoproteinemia in Rural Ugandan Children. *Lancet* II: 1047-1049, 1971

17 APR 1976



New from Academic Press

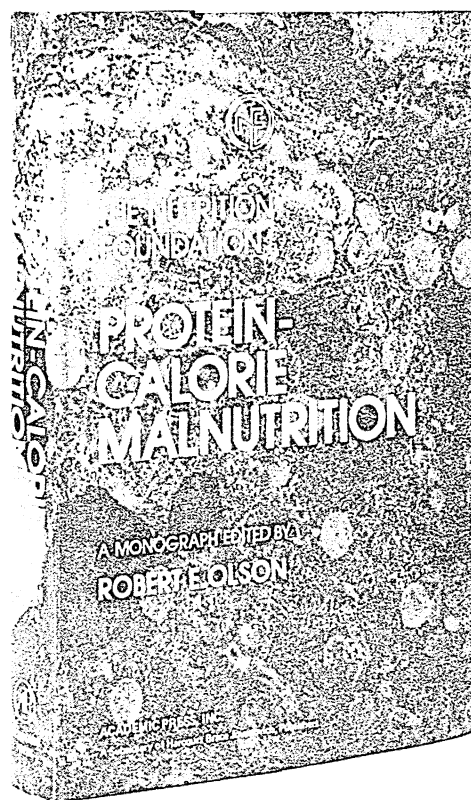
PROTEIN-CALORIE MALNUTRITION

A Nutrition Foundation Monograph

For physicians, nutritionists, public health practitioners, social workers, and medical students.

Covers all aspects of protein-calorie malnutrition (PCM), from molecular biology to epidemiology and prevention.

467 pages, \$29.50. Order from:
Academic Press, 111 Fifth Avenue,
New York, New York 10003
or your book supplier.



THE ARCHIVES OF INTERNAL MEDICINE

Volume I, 1908

Pages 349-384

THE RELATION OF IODIN TO THE STRUCTURE OF THE THYROID GLAND.

DAVID MARINE, M.D. and W. W. WILLIAMS, M.D.

[From the Laboratories of Pathology and Pharmacology of
Western Reserve University.]
Cleveland, Ohio.

INTRODUCTION.

The analyses and experiments embodied in this paper comprise the second stage of the work on the nature of goiter, viz.: the relation of iodine to the structure of the thyroid gland.

In previous papers one of us discussed the structure of the thyroid in its various phases in the attempt to formulate basic anatomic types to which the individual glands could be referred. It was found that the various types of changes are quite easily separated into four natural groups, viz.:

- (1) Normal glands.
- (2) Glandular hyperplasias, all degrees (parenchymatous hypertrophies).
- (3) Colloid glands, goiter (all degrees).
- (4) Complications supervening in any of the three preceding groups.

• • •

The general views as to the etiology of goiter may be divided into two groups: (1) that it is due to an infecting agent (miasm) and (2) that it is the manifestation of a nutritional disturbance. The former had its period of greatest development parallel with the early period of bacteriology (1850-1885). Virchow, Hirsh and, in particular, Ewald have supported this view.

The latter view, that it is a nutritional disturbance, has received its greatest support from experimental investigations. The experiments, accidental, as it were, of the elder Kocher, and Reverdin, of the total removal of the gland in man, were probably the most suggestive in the early period. The work of Munk on the importance of the thyroid gland to the animal, pointing to conclusions which were so contradictory to the prevailing views, stimulated Halsted, in the early days of aseptic surgery, to repeat them. Halsted's fundamental experiments and observations, followed by those of Horsley and Edmunds, have established several important general biologic facts, chief among which are: (1) that the gland is essential to normal body economy; (2) that partial removal is followed by compensatory hyperplasia of the remaining portion; (3) that myxedematous conditions follow the total removal of the thyroid gland alone, and (4) that it is possible to produce experimentally congenital (goiter) hyperplasia of the thyroid.

• • •

The results of all the investigations included in the above summary have tended to show (1) that the thyroid gland is an organ essential to the normal metabolic activities of the animal; (2) that it probably has a definite secretion; (3) that iodine is an essential constituent of this secretion, and (4) that the various anatomic changes occurring in the gland are results of or reactions against general nutritional disturbances rather than specifically thyroid in origin.

ANATOMIC CONSIDERATIONS.

• • •

The material for this work consists of the thyroid glands of a series of over one hundred dogs taken without discrimination as to weight, age, sex or breed from the several department laboratories. Care was taken, however, to get the thyroids as soon as possible after the dogs were brought from the street in order that the findings might with justice be taken as an index of the normal street condition. As factors which might conceivably affect the condition of the thyroid, we observed the age, sex, breed, weight and state of nutrition. Records were made of all these conditions, though as regards age and breed gross errors are probable. The thyroid glands were weighed fresh; brief descriptions of color, consistency and visible colloid were noted; a small piece for microscopic examination was then removed, and the gland reweighed and referred to Dr. Williams for iodine determination.

• • •

CONCLUSIONS.

1. Iodine is necessary for normal thyroid activity.
2. Iodine is the main index to the physiologic value of the thyroid secretion (thyroglobulin) to the body.
3. The percentage of iodine varies with the amount of colloid in the several degrees of hyperplasia; the colloid varies inversely with the degree of thyroid hyperplasia; the iodine varies inversely with the degree of thyroid hyperplasia.
4. The colloid gland (goiter) is the quiescent or normal state of a gland which has previously been a glandular hyperplasia and obeys all the biologic laws of a normal gland.
5. In the classification of thyroid changes (excluding infections and neoplasms) four major groups must be recognized, viz.: (1) normal glands, (2) colloid glands, (3) hyperplastic glands (all grades), (4) complications engrafted on any of the three preceding groups.
6. The ability of the thyroid glands to store iodine depends on the degree of glandular hyperplasia rather than on the form or mode of administration of iodine.

THE EFFECT OF PROLACTIN ON LIPOPROTEIN LIPASE ACTIVITY

Prolactin administration produces marked changes in the lipoprotein lipase activity of the mammary gland and adipose tissue of hypophysectomized lactating rats. The action of prolactin on enzyme activity is such that it diverts blood triglyceride utilization from storage to milk production. Lipoprotein lipase activity of pigeon adipose tissue and crop sac is also influenced by prolactin administration.

Key Words: lipoprotein lipase, prolactin, adipose tissue, mammary gland

The enzyme lipoprotein lipase catalyzes the hydrolysis of triglycerides which are present in the blood in the form of chylomicrons or lipoproteins. The enzyme appears to be present in most tissues closely associated with the capillary walls. The fatty acids released by its action may be utilized for energy purposes or resynthesized to triglyceride by the tissue. It is now reported by Scow and his co-workers^{1,2} that the lipoprotein lipase activity in rat adipose tissue, rat mammary gland and pigeon crop sac is markedly influenced by the level of the pituitary hormone, prolactin, circulating in the blood stream.

Previous studies from a number of laboratories showed that during lactation lipoprotein lipase activity is increased in the mammary gland and decreased in adipose tissue. The work of Zinder et al.¹ supplies evidence that these changes are probably due in large part to changes in prolactin secretion. In these studies rats that had been lactating for five to six days were hypophysectomized. One group received no treatment while a second group received subcutaneous injections of prolactin and other hormones. Assays for lipoprotein lipase activity were performed on dried defatted powders of the tissues by measuring the amount of rat chylomicron triglyceride that was converted to fatty acids. (Activity is expressed as 1 U = 1 μ mole of triglyceride hydrolyzed per hour.) Rats

in both groups received daily injections of 1 U of oxytocin to stimulate milk ejection in order to prevent the lowering of lipoprotein lipase activity that accompanies engorgement of the mammary gland. During the first 24 hours following hypophysectomy all rats showed a severe but transient diabetes and a marked body weight loss. In the following 24 hours some rats continued to lose while others gained weight. In those losing weight there was a marked depression in the lipoprotein lipase activity of adipose tissue whereas enzyme values were near normal in those animals that gained weight. In order to avoid complications from this factor only those rats which showed a weight gain during the 24 to 48 hour postoperative period were employed as controls for the hormone-injected animals.

The average lipoprotein lipase activity of the mammary gland and adipose tissue from normal nonlactating rats is 6.6 U and 10.8 U per gram respectively. In animals that have been lactating seven to eight days these values become 50.1 U and 2.4 U. If hypophysectomy is performed on the fifth to sixth day of lactation, then 48 hours following the operation the enzyme activity in the two tissues drops to values that are about those of the normal nonlactating animal. The effect of hypophysectomy on the enzyme activity of the mammary gland is rapid, a decrease of 85 percent being seen within six hours after the operation. This marked alteration in enzyme activity is not

seen, however, if bovine prolactin injections are started two hours following surgery and given every six hours for two days. (Total prolactin 2 mg daily.) Prolactin was also administered with different combinations of dexamethasone, growth hormone and thyroxine; no striking alterations were seen from those produced by prolactin alone. The supplementary hormones when administered without prolactin produced some alterations in enzyme activities, dexamethasone alone having the greatest effect. Administration of dexamethasone, however, blocked body weight gain during the second day of treatment. Therefore its action could be secondary to its effect on food intake for the reasons noted previously. Thus the marked changes seen in the lipoprotein lipase activity of adipose tissue and mammary gland of the lactating rat would appear to be chiefly due to secretion of prolactin.

The effect of prolactin on the lipoprotein lipase activity of the crop sac and adipose tissue of pigeons has also been examined by Garrison and Scow.² In these studies female pigeons were given daily intramuscular injections of 1 mg of ovine prolactin for periods of one to four days. Assays for lipoprotein lipase activity were performed 20 hours after the last injection of hormone. Administration of prolactin for four days caused an increase in enzyme activity from 17 to 177 U per gram in the crop sac and from 68 to 118 U per gram in adipose tissue. The activity in the crop sac increased mainly during the third and fourth days of hormone injection whereas in adipose tissue the increase in activity occurred only during the fourth day. No effect of prolactin was seen on the enzyme activity of the esophagus. The average increase in the weight of the crop sac during four days of hormone injection was from 1.4 to 7.2 g. Therefore the total lipoprotein lipase activity of the crop sac increased over 50-fold after four days of prolactin injections.

Prolactin thus increases the lipoprotein lipase activity of both the pigeon crop sac and the rat mammary gland. The action on

the adipose tissue of the two species is, however, different. In the case of the pigeon a definite increase in enzyme activity is seen, while in the rat a marked decrease occurs. The reason for this difference is not known. As pointed out by the authors, however, the normal level of lipoprotein lipase activity in pigeon adipose tissue is some five-fold greater than that of rat adipose tissue. Such a difference is in accord with the differences in metabolic characteristics of the adipose tissue of the two species. As shown by Goodridge and Ball,^{3,4} pigeon adipose tissue, unlike rat adipose tissue, has a low capacity to convert glucose to fatty acids. It is largely dependent on blood triglycerides for its fat stores and thus could be expected to possess a high lipoprotein lipase activity. It is also of interest to note that administration of prolactin to pigeons produces in the liver a rapid and marked increase in both its size and capacity to synthesize triglycerides from glucose. Thus in the pigeon prolactin acts to increase the supply of blood triglycerides that the adipose tissue may be called upon to store. The liver of the rat does not respond to prolactin administration in a manner similar to that of the pigeon.

The results of Scow and his co-workers indicate that the anterior pituitary gland controls lipoprotein lipase activity in the mammary gland and adipose tissue of the rat during lactation and that this control is mediated primarily through the secretion of prolactin. The manner in which prolactin acts to decrease the enzyme activity of adipose tissue, while increasing that of the mammary gland, remains to be elucidated. Thus the overall effect of prolactin is to divert fatty acids, derived from glucose or fat ingestion, from body fat stores to the mammary gland for the synthesis of milk. The question of whether prolactin is capable of inhibiting lipoprotein lipase activity in the adipose tissue of normal animals is one which remains to be answered. Studies along these lines might be rewarding to investigators concerned with obesity on the one hand and hyper-

lipidemia on the other. In any case the studies of Scow and his co-workers provide additional evidence for the important role that prolactin can play in the lipid metabolism of both rats and pigeons. □

1. O. Zinder, M. Hamosh, T.R.C. Fleck and R. O. Scow: Effect of Prolactin on Lipoprotein Lipase in Mammary Glands and Adipose Tissue of Rats. *Am. J. Physiol.* 226: 744-748, 1974

2. M. M. Garrison and R. O. Scow: Effect of Prolactin on Lipoprotein Lipase in Crop Sac and Adipose Tissue of Pigeons. *Am. J. Physiol.* 228: 1542-1544, 1975
3. A. G. Goodridge and E. G. Ball: Lipogenesis in the Pigeon: In Vivo studies. *Am. J. Physiol.* 213: 245-249, 1967
4. A. G. Goodridge and E. G. Ball: The Effect of Prolactin on Lipogenesis in the Pigeon. In Vitro Studies. *Biochemistry* 6:2335-2343, 1967

TAURINE INVOLVEMENT IN RETINAL AND HEART MUSCLE FUNCTION

Retinal photoreceptor cells degenerate in cats fed a diet low in sulfur amino acids and sulfate. The formation of taurine from sulfate appears to be an important pathway in heart and liver in many species, especially in the cat. Elevated heart taurine levels have been found in patients dying from congestive heart disease, but not in skeletal muscle of hypertensive rats.

Key Words: photoreceptor cells, casein, amino acid, retinal degeneration, taurine, methionine, cysteine, potassium

Except for the formation of taurocholic acid,¹ little information is as yet available on the function and metabolism of taurine, despite the occurrence of taurine in all vertebrate organs analyzed and the fact that it is a major amino acid in urine. Speculation has been made that taurine may be a neurotransmitter like gamma-amino-butyric acid.^{2,3} Taurine is a major amino acid in squid axons and is implicated in regulation of potassium concentration in heart cells. Other evidence suggests that both taurine and isethionic acid are involved in membrane excitability. Taurine is converted to isethionic acid via removal of the amino group, and this process has been demonstrated in dog heart slices and rat brain.

More tangible evidence for the functions of taurine has been obtained recently. This includes the reports (a) that taurine deficiency may result in retinal degeneration in the cat⁴ and (b) that an increased taurine concentration has been found in heart

muscle of hypertensive rats, with no change in skeletal muscle or brain.⁵

Hayes and co-workers⁴ noted earlier that retinal degeneration visible with an ophthalmoscope occurred within three months of feeding kittens or adult cats a diet with casein as the dietary protein. In this condition, there was a decrease in the amplitude of the cone and rod components of the electroretinogram. A time delay also appeared in the electrical response of the cone. These changes coincided with degeneration of the photoreceptor cells. Other ultrastructural changes occurred in the photoreceptor outer segments, and eventually the entire photoreceptor population degenerated.

That the retinal degeneration was related to the use of casein as the dietary protein was shown by the fact that substitution of lactalbumin or egg albumin for casein prevented or reversed the degeneration. The major difference between casein and these proteins is their higher sulfur amino acid content (168 and 181 percent of the level in casein). This information, coupled with the fact that high levels of taurine

have been found in retina of several mammalian species, led Hayes and his co-workers to investigate the concentrations of taurine and other amino acids in the retina and plasma of cats fed diets containing casein as the major protein source.

Kittens and adult cats were fed diets containing casein as 16 to 27 percent of the dietary calories. All of the animals showed retinal degeneration after these diets had been fed for three months or more. Plasma amino acid analyses showed that taurine was almost completely absent, although the levels of other sulfur amino acids were similar to values found in cats fed a commercial chow diet.

The retinal levels of taurine in the casein-fed cats decreased steadily, even before signs of retinal degeneration occurred, as indicated by decreased DNA concentration. An 84 percent decrease in retinal taurine occurred after 24 weeks whereas the decrease in DNA was only 13 percent. No significant changes occurred in the retinal levels of glutamic acid, aspartic acid, glycine or glutamine-serine in the cats fed the casein diet.

Preliminary experiments with kittens fed the casein diet supplemented with taurine, methionine or cysteine indicated that taurine supplementation appeared to prevent the retinal lesions, whereas methionine and cysteine were ineffective. These observations were based on funduscopic examination of the retina and still must be confirmed by electroretinographs and electron microscopic studies of the retinas.

The authors speculated that a deficiency of taurine could have arisen from a number of factors in the cats fed the casein diet. First, casein is relatively low in sulfur amino acids. Second, this diet was also low in sulfate, so that the potential contribution of taurine synthesis from inorganic sulfate⁶ would have been minimized. Third, the cats cannot decarboxylate appreciable amount of cysteine sulfinic acid to taurine, so that this route would have produced relatively little taurine. Other possible factors included reduced synthesis of taurine in the retina itself or reduced

uptake of taurine from the plasma. Kittens grow very rapidly and require a high protein diet. Even when the protein is supplied from sulfur amino acid-rich proteins, such as fish and liver, no taurine is found in the liver unless the dietary protein levels are above 21 percent of the diet (dry weight). Felinine, however, an isopentanol derivative of cysteine occurring in felines, still appeared in the urine, although the dietary sulfur amino acid intake was inadequate for taurine formation.

A role for taurine involvement in ion movement in muscle had been suggested by earlier work of Huxtable and Bressler.³ Subsequently, they found that rats made hypertensive by environmental stresses (loud noises, cage oscillation, etc.) had significantly higher levels of heart taurine than did unstressed rats.⁵ No increases occurred in the taurine concentration (μ moles taurine per gram of protein) in muscle and brain. Cardiac hypertrophy also occurred in the hypertensive rats. An increased ratio of taurine to protein in heart muscle was detected also in a strain of rats which developed spontaneous hypertension.

Huxtable and Bressler⁵ also measured the taurine content of the left ventricle from patients who died from congestive heart failure and in patients with death from other causes. No correlation was found between taurine concentration and the age of the subjects in either group. (Subjects under ten years old had been excluded since taurine concentrations in tissues are normally higher in young children.) The taurine concentration in the left ventricle of subjects with congestive heart failure was significantly higher than in the left ventricle of patients who died from other causes. This increase occurred when the taurine concentration was expressed as μ moles per gram of wet tissue, μ moles per gram of acid-precipitable material or μ moles per gram of protein. It was necessary to express taurine concentration in several ways since the heart tissue analyzed varied considerably in hydration, fibrous composition and percent of fat. With all three forms of estimation, how-

ever, the taurine concentration was higher in the ventricular tissue from patients who died of congestive heart failure.

In contrast, the taurine concentration of aortic tissue was no higher in patients with congestive heart failure. Control analyses showed that taurine was stable in frozen heart tissue for as long as a year. Consequently, the differences observed were not the result of the loss of taurine during storage before analysis.

Whether the elevated taurine concentration is a factor in the development of congestive heart disease, or whether it is a secondary condition remains to be determined. The occurrence of an increase is especially unusual since changes in chemical composition have been observed only rarely in heart tissue of patients dying with heart failure.

Other work by Huxtable and Bressler² showed that the highest tissue concentration of taurine in rats was in the heart, as well as the greatest uptake of ^{14}C -taurine of any organ at three days after injection of the radioactively-labeled taurine. Skeletal muscle contained the largest total amount of radioactivity at three days after injection. Maximum specific activity in heart taurine occurred five days after injection. Most of the injected taurine in tissues was in the soluble portion of the cytoplasm, but some occurred in the microsomal fraction, with very little in mitochondria.

A small amount of ^{14}C -isethionic acid was found in all organs, but this does not imply formation of isethionic acid in that organ. Uptake and distribution of ^3H -labeled isethionic acid was tested after intraperitoneal injection. About 80 percent of the injected compound was excreted in the urine within 24 hours after injection. No radioactivity was detected in heart taurine. Similar results on tissue uptake of ^{35}S -taurine in rats were obtained by Spaeth and Schneider.⁷ In the liver, the turnover of taurine may be related to taurocholic acid formation.

As Hayes and co-workers pointed out, the casein diet fed to cats was low in inorganic sulfate as well as in sulfur amino

acids. Recent work by Martin and co-workers⁶ showed the potential importance of the synthesis of taurine from sulfate and serine in the rat. Their results indicate that this pathway is important in the maintenance of taurine levels in heart, muscle and brain, especially in pyridoxine-deficient animals in which taurine synthesis from cysteine via cysteine sulfinic acid was reduced. Early work considered that significant synthesis of taurine from sulfate did not occur in mammals, although this pathway is a significant one in chickens. To demonstrate *in vivo* the presence of the enzyme system catalyzing the synthesis of taurine from L-serine and 3'-phospho-adenosine-5'-phosphosulfate (PAPS), rats were given $^{35}\text{SO}_4$ by stomach tube and sacrificed six hours later. The specific activity of liver taurine increased with time, whereas the total amount of taurine in the liver stayed constant.

The diet fed the rats was high in methionine (supplemented with 0.3 percent D-methionine) since previous experiments with chicks showed that high methionine diets increased the conversion of sulfate to taurine. Thus, it was possible that the conversion of sulfate to taurine was regulated by the level of intake of sulfur amino acids. The level of PAPS and serine depend upon the methionine and cysteine levels of the diet. Cysteine inhibits ATP-sulfurylase, the first enzyme required for synthesis of PAPS. Other experiments of Martin and co-workers showed that cysteine, homocysteine and S-adenosylmethionine were effective *in vitro* inhibitors of the chick liver enzyme system catalyzing the formation of taurine from L-serine and PAPS.⁸ L-methionine was also inhibitory but to a lesser extent than cysteine which was effective at very low concentrations. Isethionic acid was only mildly inhibitory. D-methionine at lower concentrations caused a 20 percent increase in activity.

Experiments with pyridoxine-deficient rats showed that the concentration of taurine in the heart remained constant in the deficient rats, although the liver taurine concentration decreased.⁹ The activity of

the enzyme system converting sulfate to taurine remained constant in the liver of deficient rats, while the activity of liver cysteine sulfinic acid decarboxylase decreased. The amount of taurine formed from $^{35}\text{SO}_4$ increased in both the heart and the liver with the duration of pyridoxine deficiency.

The report that taurine can prevent the loss of potassium in the heart led to experiments on the effect of potassium intake on the formation of taurine from sulfate.¹⁰ Rats were pair-fed on diets containing 0.1, 0.2 or 0.4 percent potassium for four weeks. They were then injected with radioactive sulfate and sacrificed four hours after injection. The activity of the enzyme system catalyzing the synthesis of taurine from sulfate increased with the potassium level of the medium in both heart and liver preparations. Activity decreased after dialysis and was restored by the addition of potassium to the medium, provided that the dialysis was not too prolonged. The activity of this enzyme system was not restored by the addition of ammonium, sodium, lithium, magnesium or calcium ions.

Heart taurine was highest with 0.2 percent potassium in the diet, and lower at the 0.1 percent and 0.4 percent levels. The change in taurine level with dietary potassium was much more pronounced in the heart than in the liver. The activity of the enzyme system in liver and heart was also greater at the 0.2 percent potassium level and lower at the other two concentrations. The specific activity decreased from 750 to 570 in both tissues. The incorporation of $^{35}\text{SO}_4$ into taurine was also affected by the dietary potassium level, with the highest specific activities at the 0.2 percent potassium level, as would be expected from the effects of dietary potassium on the activity of the enzyme system. Thus, the ability of the heart and liver to form taurine from sulfate is affected by the dietary potassium level.

A survey of other species for the ability to synthesize taurine from sulfate showed that this pathway occurs in a variety of

mammalian species and in several organs in each species.¹¹ The species tested included the cat, dog, guinea pig, hamster, monkey, mouse, rabbit and sheep, as well as the rat. The specific activity of the enzyme was similar in heart and liver preparations from each species tested; the activities for heart and liver were quite similar in all of the species. The activities in the cat were no higher than in other species although the ability of the cat to decarboxylate cysteine-sulfinic acid and cysteic acid is low. The cat, man and horse are the three species tested in which the ability to produce taurine from cysteine in liver is limited.¹²

All of these results now provide more definite evidence for physiological roles of taurine and for regulation of its synthesis. Furthermore, these results again demonstrate the value of using a variety of animal models to determine metabolic functions of compounds important in human physiology. □

1. Importance of Bile Acids and an Intact Small Intestine for Fat Absorption. *Nutrition Reviews* 26: 85-87, 1968
2. R. Huxtable and R. Bressler: Taurine and Isethionic Acid: Distribution and Interconversion in the Rat. *J. Nutrition* 102: 805-814, 1972
3. R. Huxtable and R. Bressler: Effect of Taurine on a Muscle Intracellular Membrane. *Biochim. Biophys. Acta* 323: 573-583, 1973
4. K. C. Hayes, R. E. Carey and S. Y. Schmidt: Retinal Degeneration Associated with Taurine Deficiency in the Cat. *Science* 188: 949-951, 1975
5. R. Huxtable and R. Bressler: Taurine Concentrations in Congestive Heart Failure. *Science* 184: 1187-1188, 1974
6. W. G. Martin, N. L. Sass, L. Hill, S. Tarka and R. Truex: The Synthesis of Taurine from Sulfate. IV. An Alternate Pathway for Taurine Synthesis by the Rat. *Proc. Soc. Exp. Biol. Med.* 141: 632-633, 1972
7. D. G. Spaeth and D. L. Schneider: Turnover of Taurine in Rat Tissues. *J. Nutrition* 104: 179-186, 1974
8. L. J. Hill and W. G. Martin: The Synthesis of Taurine from Sulfate. V. Regulatory Modi-

fiers of the Chick Liver Enzyme System. *Proc. Soc. Exp. Biol. Med.* 144: 530-533, 1973

9. W. G. Martin, R. C. Truex, S. Tarka, W. Gorby and L. Hill: The Synthesis of Taurine from Sulfate. VI. Vitamin B₆ Deficiency and Taurine Synthesis in the Rat. *Proc. Soc. Exp. Biol. Med.* 147: 835-838, 1974
10. W. G. Gorby and W. G. Martin: The Synthesis of Taurine from Sulfate VIII. The Effect of

Potassium. *Proc. Soc. Exp. Biol. Med.* 148: 544-549, 1975

11. W. G. Martin, C. R. Truex, S. M. Tarka, L. J. Hill and W. G. Gorby: The Synthesis of Taurine from Sulfate. VIII. A Constitutive Enzyme in Mammals. *Proc. Soc. Exp. Biol. Med.* 147: 563-565, 1974
12. J. G. Jacobsen and L. H. Smith, Jr.: Biochemistry and Physiology of Taurine and Taurine Derivatives. *Physiol. Rev.* 48: 424-511, 1968

BIHORMONAL CONTROL OF KETOGENESIS

In the liver of fed rats enhanced fatty acid oxidation and ketogenesis develop quickly under the influence of either anti-insulin antibodies or glucagon.

Key Words: ketogenesis, glucagon, insulin antibodies, carnitine acyltransferase

The changes occurring in lipid and carbohydrate metabolism during starvation and refeeding have been studied extensively, but the controls regulating them have not been completely identified.¹ A recent contribution by McGarry, Wright and Foster² points strongly to the possibility that carnitine acyltransferase, catalyzing transfer of long-chain fatty acids across the mitochondrial membrane, is subject to control by insulin and glucagon as is carbohydrate metabolism.

These experiments were conducted with 100 g male Sprague-Dawley rats said to be fed a diet containing 58.5 percent sucrose, 21 percent casein and all necessary vitamins and minerals and which contained less than 1 percent fat. All experiments were conducted between 7 and 8 A.M. When fasted rats were used they were deprived of food for 24 hours.

Venous and arterial catheters were installed under light ether anesthesia. After recovery, heparin was infused, followed by venous infusion of guinea pig serum containing insulin antibodies, glucagon or neither agent. Control and serial arterial blood samples were taken for analysis during the experimental period. Finally pentobarbital was given intraperitoneally and the liver was removed for perfusion to determine ketogenic capacity or was quickly

frozen in liquid N₂, followed by analysis for glycogen. In some cases blood was obtained from the abdominal aorta for analysis of plasma free fatty acids, insulin and glucagon.

Livers were perfused with a recirculating medium of 20 percent aged, dialyzed human red cells in Krebs bicarbonate buffer containing bovine albumin. Oleic acid, bound to albumin, was used in the medium except when [1-¹⁴C]-octanoylcarnitine was used. In this instance the carnitine ester was added after 15 minutes of perfusion with no added fatty acid.

Blood and perfusion media were analyzed for glucose, ketones, free fatty acids, insulin, glucagon and ¹⁴C activity as required by standard procedures previously employed by these investigators.

Anti-insulin serum given to fed rats produced a strong hyperglycemia and ketonemia. Ketones were as high at two hours as in rats fasted for 24 hours. Glucagon had essentially no effect on either parameter, nor did the control serum. Liver glycogen was reduced rapidly both by insulin antibodies and by glucagon. Plasma free fatty acids were increased by insulin antibodies but not by glucagon.

Hepatic ketogenesis from oleate as measured in perfused liver increased steadily under the influence of either insulin antibodies or glucagon and in three hours attained levels 75 percent as high as those

of livers from 24-hour fasted animals. Control serum given intravenously or the anti-insulin serum or glucagon added directly to the liver perfusion medium did not affect liver ketogenesis. Nonetheless, glucagon did enhance net glucose production markedly when added to the perfusion medium.

Since glucagon increased ketogenic capacity of the liver but did not increase plasma ketones, whereas insulin antibodies increased both parameters, it was hypothesized that glucagon did not increase the rate of delivery of free fatty acids to the liver. Confirmation of this was obtained by infusion of a fat emulsion into glucagon-treated, fed rats and noting an acute elevation of plasma ketones, not noted without glucagon treatment although plasma free fatty acids increased similarly in both groups.

Previous investigations by this group³ suggested that the carnitine acyltransferase reaction is the rate-limiting step in fatty acid oxidation and is the primary site for activation of this process in ketotic liver. In the present experiments the effects of anti-insulin serum and glucagon on this reaction were tested, using [1-¹⁴C]-octanoylcarnitine as a substrate, since it requires activity of carnitine acyltransferase II for its oxidation. Thus, livers were perfused in the presence of this compound (without oleate) for 15 minutes and then isotopically labeled octanoylcarnitine was added to the medium and the quantity and specific activity of total ketones formed were measured. Either anti-insulin serum or glucagon increased ketone production from the labeled ester by fivefold in three hours, an increase 70 percent of that attained by a 24-hour fast and similar to that observed when oleate was the substrate.

In the experiments described earlier glucagon was infused at a high level to maximize the response obtained. Subsequently some of these experiments were repeated, using 0.5 percent of the previous dose. This resulted in a doubling of the normal glucagon level in the plasma and a 23 percent depression in circulating insulin. After

three hours of infusion the ketogenic capacity of the liver had increased threefold, oxidation of long-chain fatty acids and of octanoylcarnitine had been stimulated, and liver glycogen had been depressed to the same extent as in one hour with the larger amount of glucagon or of insulin antibodies.

These studies show that enhancement of hepatic long-chain fatty acid oxidation and ketogenesis that occurs in the rat after six hours of starvation may be induced in about one hour by infusion into fed animals of insulin antibodies or glucagon, thus indicating an abrupt shift from a nonketogenic to a ketogenic profile. Generation of a ketotic liver with anti-insulin serum was expected, since this agent produced a generally ketotic state in vivo. Less expected was the equally ketogenic liver produced by glucagon, since the whole animal appeared to be nonketotic. Presumably this was a result of increased insulin activity stimulated by glucagon-induced hyperglycemia. The increased insulin would inhibit lipolysis and decrease free fatty acid transport to liver and hence reduce ketone body production. This thought is supported by the ketotic state induced by the combined treatment with glucagon and a fat emulsion. Thus two requirements for development of a ketotic state exist. An accelerated mobilization of free fatty acids to the liver and a metabolic pattern in the liver stimulating fatty acid oxidation. Insulin deficiency seems not a requirement, but rather a change in the ratio of glucagon to insulin as seen in the final experiment with relatively low levels of glucagon causing only a 23 percent depression in circulating insulin. Thus, hepatic fatty acid metabolism and ketogenesis may be under bihormonal control as is glucose homeostasis.

The observed proportional stimulation of hepatic long-chain fatty acid oxidation and oxidation of octanoylcarnitine, whether induced acutely by insulin antibodies or glucagon or more gradually by starvation, suggests that the common denominator of all three situations is activa-

tion of carnitine acyltransferase II. The data do not permit speculation about carnitine acyltransferase I.

These experiments do provide new evidence on control of hepatic ketogenesis, although they do not yet uncover the mechanism whereby the enzyme systems are regulated. Since hepatic ketogenesis was seen to be inversely correlated with hepatic glycogen levels, it appears likely that some component of hepatic carbohydrate metabolism plays an important role. The aphorism that "Fats burn in the flame of the carbohydrates" is still alive! □

1. The Time Course of Changes in Carbohydrates and Lipid Metabolism in the Rat as Caused by Starvation and Refeeding. *Nutrition Reviews* 31:222-223, 1973.
2. J. D. McGarry, P. H. White and D. W. Foster. Hormonal Control of Ketogenesis. Rapid Activation of Hepatic Ketogenic Capacity in Fed Rats by Anti-Insulin Serum and Glucagon. *J. Clin. Invest.* 55:1202-1209, 1975
3. J. D. McGarry and D. W. Foster. The Metabolism of (-)- Octanoylcarnitine in Perfused Livers from Fed and Fasted Rats. Evidence for a Possible Regulatory Role of Carnitine Acyltransferase in the Control of Ketogenesis. *J. Biol. Chem.* 249:7984-7990, 1974

INDEX OF NUTRITION EDUCATION MATERIALS

The Index of Nutrition Education Materials has been prepared by the Office of Education of the Nutrition Foundation to assist teachers and the general public in acquiring useful teaching aids and educational materials.

The Index lists more than 1,000 booklets, pamphlets, audio-visual aids and lists of recommended books available from federal agencies, professional societies, and educational organizations or foundations. Other sources for education material are included.

Gives addresses of important local contacts and sources in each state.

Source, subject matter, readership level and description are given for each publication in color-coded sections for easy reference. \$5.00 postpaid

Order from:

OFFICE OF EDUCATION
THE NUTRITION FOUNDATION
888 Seventeenth Street, N.W.
Washington, D. C. 20006

Letter to the Editor

International Comparison of Body Measurements in Boys

Sir: The review of our paper "Selected Body Measurements in Boys Ages 6-11 Years from Six Villages in Southern Tunisia: An International Comparison" (*Nutrition Reviews* 33: 235-236, 1975), raised some questions. In the following, we will try to answer one and comment on two.

1. Age: "The authors have not mentioned how the ages of their subjects were assessed." This is not correct. On page 472 of our paper, under Methodology is stated: "The ages of the boys were obtained from school records which showed the age as stated on the birth certificate." In Egypt, ages were obtained in a similar way. In India, the ages of 25 percent of the sample of 44,000 children were verified through an interview with the parents based on documentary evidence like authentic home records, baptismal verification certificates, birth registers, etc. (see page 6 of the reference by Mr. McDowell et al.). Height and weight means of the age verified cases were almost identical with those for the total sample. One may be justified to assume that ages in the three groups are quite reliable in contrast to samples in many other developing countries. In Tunisia, newborn children are usually registered within one month after birth, but sometimes later. Any error would, therefore, be on the plus side; the child may be actually older than the age given on the certificate.

2. Comparability of highest economic group in Tunisia to the average in USA: One can argue about the comparability of the two groups in terms of socio-economic level. The reviewer refers to a study from India showing the growth rate of Indian

children from the highest economic group to be very similar to that of American children in the relevant ages (6-12). The reviewer did apparently not question the comparability of those two groups. Was it because they showed practically no differences? I would interpret these findings in favor of genetic factors explaining the relatively small differences between the rich Tunisians and the average Americans because the poor Indian boys were even below the poor Tunisians, in other words, the gap between the poor and the rich in India was still greater than in Tunisia. In other words, the genetic potential of the Indian boys appears to be greater than that of the Tunisian boys. However, I would admit that the true genetic potential of the Tunisian boys may be greater than assessed from this small sample of rich boys in Tunisia.

3. In regard to the "completely ignoring of calorie deficiency as the main nutritional constraint on the growth of children in the developing countries": It was mentioned that the south had consistently greater percentages of people who consumed amounts of calories and other essential nutrients below the lowest acceptable value than the other regions. It is agreed, however, that the possibility of calorie deficiency should have been emphasized more strongly.

Frank W. Lowenstein, M.D.
Nutrition Advisor
National Center for Health
Statistics, DHEW
Rockville, Maryland 20852

Recent Books

Protein and Nutrition Policy in Low-Income Countries, by F. Aylward and M. Jul. Published by Charles Knight and Company Ltd., London, 1975. Pp. 150. Price £ 2.50.

1972 Evaluations of Some Pesticide Residues in Food: The Monographs. Published by Food and Agriculture Organization, Rome, 1973. Available from: Unipub, Box 433, Murray Hill Station, New York, New York 10016. Pp. 587. Price \$10.00.

Chemicals and Health. Report of the Panel on Chemicals and Health of the President's Science Advisory Committee. Published by Science Advisory Committee. Published by Science and Technology Policy Office, National Science Foundation, 1973. Available from: Superintendent of Documents, U. S. Government Printing Office, Washington, D. C. 20402. Pp. 211. Price \$2.75.

Nickel. Published by National Academy of Sciences/National Research Council, 1975. Publication No. ISBN 0-309-02314-9. Available from: Printing and Publishing Office, National Academy of Sciences, 2101 Constitution Avenue, Washington, D. C. 20418. Pp. 277. Price \$10.75.

Lactic Acid Bacteria in Beverages and Food. J. G. Carr, C. V. Cutting and G. C. Whiting, Editors. Fourth Long Ashton Symposium, 1973. Published by Academic Press, 111 Fifth Avenue, New York, New York 10003 (1975). Pp. 415. Price \$28.50.

Proceedings of the Western Hemisphere Nutrition Congress IV. P. L. White and N. Selvey, Editors. Published by Publishing Sciences Group, Inc., Acton, Massachusetts, 1975. Pp. 407.

Panic in the Pantry: Food Facts, Fads and Fallacies, by E. M. Whelan and F. J. Stare. Published by Athenium, New York, 1975. Pp. 231. Price \$8.95.

The Nutrition Crisis: A Reader, by T. P. Labuza. Published by West Publishing Company, New York, 1975. Pp. 512. Price \$8.95 (paperback).

FDA Toxicology Advisory Committee Formed

The Food and Drug Administration recently announced the membership of its newly-formed Toxicology Advisory Committee.

The purpose of the committee is to advise FDA on matters relating to the safety of chemicals present in foods, drugs, cosmetics and medical devices. The committee will conduct independent scientific reviews of potentially toxic chemicals and methods for toxicity testing of such materials. Its membership is composed of experts in the fields of toxicology, pharmacology, carcinogenesis testing and related subjects. □

Meeting Announcement

The 6th Argentine Congress of Nutrition will take place in Buenos Aires, October 3-7, 1976. The Congress will cover important subjects on basic and clinical nutrition, nutrition education and food technology in relation to nutrition. Participants will include physicians, dietitians, nutritionists, food technologists, sociologists, economists and experts in agricultural and food industries.

All requests for information should be directed to the Secretary General, 6to. Congreso Argentino de Nutricion, Cangallo 2049 (Piso 10, Of. 78), Buenos Aires, Argentina. □

Special Notice

All subscription inquiries, renewal requests and change of address notices for NUTRITION REVIEWS should be sent to:

The Nutrition Foundation
Office of Education
888 Seventeenth Street, N.W.
Washington, D. C. 20006

STATEMENT OF OWNERSHIP, MANAGEMENT AND CIRCULATION

(Act of August 12, 1970: Section 3685. Title 39
United States Code)

1. Title of publication NUTRITION REVIEWS
2. Date of filing 10/1/75
3. Frequency of issue MONTHLY
4. Location of known office of publication (Street, city, county, state, ZIP code) (Not printers) 489 Fifth Avenue, New York, New York 10017
5. Location of the headquarters or general business offices of the publishers (Not printers) 489 Fifth Avenue, New York, New York 10017
6. Names and addresses of publisher, editor, and managing editor
Publisher (Name and address) THE NUTRITION FOUNDATION, Inc., 489 Fifth Avenue, New York, New York 10017
Editor (Name and address) D. Mark Hegsted, Harvard School of Public Health, 665 Huntington Avenue, Boston, Mass. 02115
Manager Editor —
7. Owner (If owned by a corporation, its name and address must be stated and also immediately thereunder the names and addresses of stockholders owning or holding 1 percent or more of total amount of stock. If not owned by a corporation, the names and addresses of the individual owners must be given. If owned by a partnership or other unincorporated firm, its name and address, as well as that of each individual must be given.) The Nutrition Foundation, Inc., 489 Fifth Avenue, New York, New York 10017
8. Known bondholders, mortgagees, and other security owning or holding 1 percent or more of total amount of bonds, mortgages or other securities (if there are none, so state) NONE
9. For optional completion by publishers mailing at the regular rates (Section 132.121, Postal Service Manual)—Not completed
10. For completion by nonprofit organizations authorized to mail at special rates (Section 132.122, Postal Manual)
The purpose, function, and nonprofit status of this organization and the exempt status for Federal income tax purposes ☐ Have not changed during preceding 12 months

11. Extent and nature of circulation	Average no. copies each issue during preceding 12 months	Actual number of copies of single issue published nearest to filing date
A. Total no. copies printed (Net Press Run)	8,824	7,800
B. Paid circulation		
1. Sales through dealers and carriers, street vendors and counter sales		
2. Mail subscriptions	7,560	6,669
C. Total paid circulation	7,560	6,669
D. Free distribution by mail, carrier or other means; samples, complimentary, and other free copies	235	235
E. Total distribution (Sum of C and D)	7,795	6,904
F. Office use, left-over, unaccounted, spoiled after printing	1,029	896
G. Total (Sum of E & F—should equal net press run shown in A)	8,824	7,800

(Signature of editor, publisher, business manager, or owner)

I certify that the statements made by me above are correct and complete.
/s/ RICHARD M. STALVEY, VICE PRESIDENT, The Nutrition Foundation, Inc.

The Role of Dietary Carbohydrates in Plaque Formation and Oral Disease

by Albert T. Brown, Ph.D.

Dental caries and periodontal disease affect a large percent of this nation's population and have a severe impact on the total health picture of the individuals involved. It has been known for some time that the accumulation of dental plaque is intimately associated with the frequency and incidence of dental caries. Recently, the correlation of plaque formation with periodontal disease has also been established.¹⁻⁶ Dental plaque is generally considered to be an adherent, gelatinous material which covers the teeth and is composed of a high population density of oral microorganisms ($\cong 4.0 \times 10^{11}$ cells per gram wet weight) and an extrabacterial matrix consisting in part of bacterial extracellular polysaccharides, leukocytes, salivary glycoproteins, water and epithelial cell remnants. It is firmly established that the metabolic activity of dental plaque is mainly due to its microbial content.⁷⁻⁹

Dietary carbohydrates are of primary importance in leading to the colonization of microorganisms on the tooth surface(s) and they permit plaque microorganisms to sustain their life functions even under periods of nutritional stress. It is the accumulation of large numbers of oral bacteria in dental plaque, coupled with

their continuous metabolic activities which lead to dental caries and periodontal disease.

Current evidence supports the belief that sucrose is of central importance in plaque formation and metabolism by oral microorganisms.⁹⁻¹⁵ It is important to stress, however, that the importance of other dietary carbohydrates cannot be overlooked since they too contribute to plaque formation and support the life functions of certain components of the oral microflora.^{9,14,16-22} At least four aspects of dietary carbohydrate utilization by oral microorganisms collectively contribute to their disease potential through their role in the formation of dental plaque and through the metabolic activity of various components of the plaque microflora which yield a number of bacterial products that directly interact with oral hard and soft tissues or elicit an appropriate host response.

The four important areas of dietary carbohydrate metabolism are their conversion to adhesive bacterial extracellular polymers, bacterial extracellular storage polysaccharides, bacterial intracellular storage polysaccharides, and their utilization as a fermentable energy (ATP) source by plaque microorganisms. Perhaps the most germane to virulence is the utilization of carbohydrates for the generation of energy necessary to support the biosynthetic processes of oral bacteria since all common dietary carbohydrates are readily utilized in this manner by a number of the microbial components of dental plaque. The interrelationship of these four aspects of carbohy-

Dr. Brown is Associate Professor in the Department of Oral Biology at the University of Kentucky College of Dentistry, Lexington, Kentucky 40506.

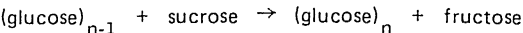
limited number of reprints of this article may be obtained from the author. THERE ARE NO REPRINTS OF UNSIGNED REVIEWS.

drate metabolism in plaque formation and oral disease is schematically represented in Figure 1.

Production of Adhesive Bacterial Polymers

The production of adhesive extracellular polymers by oral microorganisms which allow them to colonize oral hard surfaces has been most thoroughly studied in streptococcal species which are major constituents of dental plaque.^{1,8,23} One class of extracellular polysaccharides produced by these organisms is dependent upon the metabolism of exogenous sucrose by enzymes designated as dextransucrases or glucosyltransferases.^{2,4-28} The glucosyltransferases are found in the culture supernatants of oral streptococci such as the caries conducive group, *Streptococcus mutans*,²⁹⁻³⁴ and are also found on the cell surface of these organisms. Glucosyltransferases catalyze the conversion of sucrose to free fructose and a glucose poly-

mer designated as dextran or glucan as shown below.³⁵⁻⁴⁰



At least two classes of glucans are formed from sucrose by the action of the glucosyltransferase enzymes. One of these classes is of high molecular weight, cell-associated and relatively insoluble. In contrast, the other main class of glucans is found in the culture supernatants or the interbacterial matrix of plaque and is soluble. Current evidence supports that the high molecular weight, cell-associated, insoluble glucans are determinants of the adherence of oral streptococci to hard tooth surfaces^{2,8,41-44,89} whereas extracellular, high molecular weight, soluble glucans probably participate in the cohesion or agglutination of certain oral streptococcal species.^{4,5}

Extracellular polymers of fructose called levans or fructans are also synthesized from

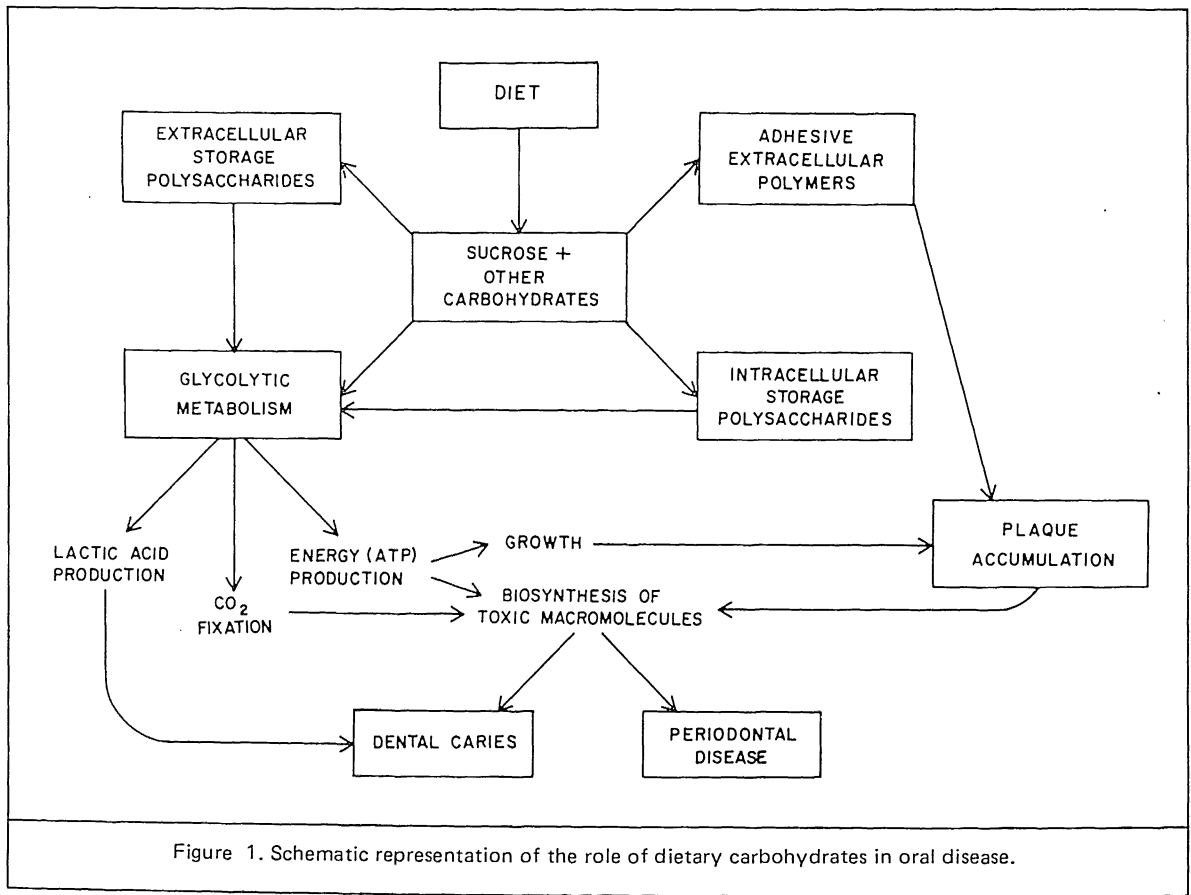
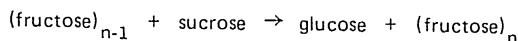


Figure 1. Schematic representation of the role of dietary carbohydrates in oral disease.

sucrose by a variety of plaque microorganisms particularly by members of the genus *Streptococcus*.^{4,6-53} The production of fructans from sucrose is catalyzed by cell-associated and/or extracellular enzymes called levansucrases or fructosyltransferases which catalyze the reaction shown below.^{54,55}



Most fructans are highly soluble and are thought to reside primarily in the culture supernatant or in the interbacterial matrix of plaque. Recent evidence, however, has suggested that small amounts of insoluble fructans may contribute to the ability of oral *S. mutans* strains to form plaque in vitro.^{28,43,44} The role of glucans and fructans as nutritional extracellular storage polysaccharides will be discussed later.

Although not as thoroughly studied, dietary carbohydrates other than sucrose are precursors of adhesive bacterial extracellular polymers and may play a role in the accumulation of certain organisms on the tooth surface. For instance, members of the genus *Actinomyces* are commonly found in dental plaque, and *Actinomyces viscosus* and *Actinomyces naeslundii* form plaque both in vivo⁵⁶ and in vitro.⁵⁷ These organisms have been implicated as etiological agents of periodontal disease,⁵⁸⁻⁶¹ root surface⁶² and coronal caries.⁶³⁻⁶⁵ In contrast to oral streptococcal strains, oral strains of *A. viscosus* and *A. naeslundii* produce no cell-associated or extracellular glucans from sucrose and their ability to form plaque in vivo and in vitro is not dependent upon the presence of sucrose.^{56,57} These organisms are able to adhere to hard surfaces in the presence of a number of carbohydrates and the plaque-forming ability of these *Actinomyces* species appears to be dependent upon the production of an adhesive polysaccharide which can be produced from a number of carbohydrate precursors.⁶⁶

Central Energy Role of Dietary Carbohydrates

Although dietary carbohydrates are precursors of bacterial extracellular polysac-

charides which permit colonization of the tooth surface, perhaps their most important role in oral disease is their ability to serve as fermentable energy sources for oral microorganisms since without energy production an organism can neither support its biosynthetic processes nor remain viable in the oral environment. All common dietary carbohydrates can be metabolized by a number of microorganisms indigenous to dental plaque and it appears that the energy-yielding metabolism of exogenous carbohydrates by the microbial components of dental plaque contributes to their disease potential in the following three distinct ways.

Contribution of Energy Production to Microbial Accumulation

The intracellular, energy (ATP)-yielding metabolism of oral microorganisms contributes to their accumulation in dental plaque by supporting the biosynthetic events leading to growth. Although the growth rate of microorganisms in plaque does not approach that observed in laboratory cultures,^{67,68} the contribution of energy-dependent microbial growth, especially in young dental plaque, cannot be overlooked in leading to the accumulation of organisms at various sites in the oral environment. Since a broad spectrum of carbohydrates serve as potential energy sources for most oral bacteria, their role in microbial accumulation (plaque formation) is of major importance.

Energy production from dietary carbohydrates contributes to plaque formation in a manner more indirect than sustaining growth. The synthesis of the enzymes which produce adhesive bacterial extracellular polymers is strictly dependent upon the energy derived from the metabolism of carbohydrates which are ultimately derived from the diet. The turnover of bacterial extracellular enzymes in plaque make their continuous synthesis an absolute necessity if they are to play a significant role in plaque formation.

Derivation of Toxic Fermentation Products from Energy Metabolism

The metabolic activity of dental plaque is a summation of the activities of its var-

ious microbial components. Since dental plaque is considered to be an essentially anaerobic environment, the utilization of exogenous carbohydrates must be done by fermentative mechanisms. A number of low molecular weight fermentation products, generally acidic in nature, which have the potential to damage oral tissues, are produced by whole plaque samples and by pure cultures of various oral bacteria.^{5,6} The fermentation product most universally produced from dietary carbohydrates by plaque microorganisms is lactic acid.^{6,9-72} This organic acid is generally considered to be the major contributor to the acidic pH of dental plaque^{73,75} especially after its exposure to exogenous carbohydrates. In addition to being an ecological determinant by contributing to low plaque pH, lactic acid is thought to play a major role in the etiology of oral disease by participating in the demineralization of enamel and cementum in coronal and root surface caries.

Energy-dependent Biosynthesis of Toxic Macromolecules

A number of macromolecules produced by oral microorganisms have been implicated as having a role in dental caries and periodontal disease. The biosynthesis of these toxic substances by plaque microorganisms at levels sufficient to damage oral tissues or elicit an appropriate host response is generally dependent upon the production of energy from carbohydrate fermentation. These macromolecules produced by oral microorganisms include a number of enzymes which collectively have the potential to degrade the organic components of enamel and dentin, disrupt the intercellular matrix of the oral epithelium, destroy connective tissue components, alter cell surfaces and cellular permeability, disrupt cell-cell adhesion, and initiate an acute inflammatory response.^{5,6,75,76} Other nonenzymatic macromolecules produced by oral bacteria also have the potential to initiate an inflammatory response. These include endotoxins produced by a number of gram-negative oral bacteria, peptidoglycans, chemotactic factors and other bacterial somatic antigens.^{5,6,77-79} It is again

important to stress that the energy required for the production of these toxic macromolecules comes from the catabolism of carbohydrates by the microorganisms in dental plaque. A list of some of the toxic macromolecules produced by plaque microorganisms and their potential role in oral disease is shown in Table 1.

Importance of Bacterial Extracellular Polymers as Energy Sources

Bacterial extracellular polysaccharides derived from exogenous carbohydrates compose a significant portion of the inter-bacterial matrix of dental plaque. In addition to these polymers playing a major role in the attachment of oral microorganisms to the hard tooth surfaces and enabling certain oral bacterial to cohere or agglutinate, they may also have a nutritive and ecological role within dental plaque by serving as fermentable energy sources which support the biosynthetic activities of plaque microorganisms during periods where dietary carbohydrates are unavailable.

Plaque microorganisms other than streptococci have been reported to make extracellular glucan-like polymers. For instance, an oral strain of *Lactobacillus casei* has been studied which produces large amounts of extracellular glucans from a number of carbohydrates in addition to sucrose.⁸⁰ Gibbons and Banghart have also shown that a cariogenic strain of *Lactobacillus acidophilus* produced an extracellular glucan antigenically similar to that produced by caries-conducive streptococci.⁸¹ It appears that glucan synthesis in plaque is not an entirely irreversible process. Manly et al. reported glucanase activity in whole plaque samples.⁸² Glucanase has also been reported to be produced by oral strains of *Streptococcus mitis*⁸³ and a number of other plaque microorganisms including *S. mutans*.⁸⁴⁻⁸⁶ Consequently, the enzymatic potential is present in plaque to catabolize glucans and to use the glucose monomers of these polymers as an energy source.

Plaque fructans produced by a number of plaque microorganisms, particularly members of the genus *Streptococcus*, ap-

Table 1	
Some Macromolecules Synthesized by Plaque Microorganisms Which Have a Potential Role in Dental Caries and Periodontal Disease	
Macromolecule	Potential Disease Role
Enzymes	
1. Proteases	Collectively degrade organic components of enamel and dentine, destroy intercellular matrix, degrade amorphous ground substance and connective tissue, alter cell surfaces, alter cell-cell adhesion and cellular permeability.
2. Hyaluronidases	
3. Neuraminidases	
4. Collagenase	
5. Chondroitin Sulfatase	
6. Glycoside Hydrolases	
7. Esterases	
Mediators of Inflammation	
1. Proteases	Collectively lead to periodontal destruction by directly eliciting an inflammatory response or inducing an allergic inflammatory response.
2. Endotoxins	
3. Peptidoglycans	
4. Chemotactic Factors	
5. Somatic Antigens	

pear to be much more labile than plaque glucans and may play an even more significant role in the nutrition and ecology of dental plaque.^{9,87} A number of oral microorganisms have been found which produce high levels of enzymes called levanases or fructanases.⁸⁸⁻⁹² These enzymes serve to degrade fructans to lower molecular weight fructose polymers or free fructose which can then be utilized as a fermentable energy source by a number of plaque bacteria.¹⁶⁻¹⁸

Bacterial extracellular polysaccharides other than glucans and fructans may be utilized by oral bacteria and hence serve as nutritional and ecological determinants. Oral *S. mutans* strains and species of lactobacilli are able to metabolize an amylopectin type extracellular polymer produced by certain members of the genus *Neisseria*, and grow much faster on this polysaccharide than on sucrose.⁹¹ Since *Neisseria* compose a significant percent of the oral microflora which colonize a clean tooth surface, the neisserial extracellular polysaccharides may be of major importance in the early stage of plaque formation.^{23,93}

Importance of Intracellular Storage Polysaccharides as Energy Sources

In addition to producing extracellular polysaccharides from dietary carbohydrates, which have an adhesive and/or nutritional role, many plaque microorganisms studied have the potential to synthesize intracellular polysaccharides from a variety of exogenous dietary carbohydrates.⁹⁴⁻⁹⁸ For instance, *L. casei*,⁹⁹ *S. mitis*^{100,101} and several strains of *S. mutans* have been shown to produce large amounts of intracellular polysaccharide material from a number of fermentable sugars. The intracellular polymers isolated from oral bacteria are generally of the amylopectin type and are probably synthesized as described by Nikaido and Hassid.¹⁰³ The role of these intracellular polysaccharides is probably similar to the nutritional role of extracellular bacterial polysaccharides in that they serve as reserve energy sources allowing oral microorganisms to sustain their biosynthetic activities over extended periods of time, even under periods of nutritional stress such as the absence of dietary carbohydrates during fasting.

Central Role of Glycolysis in Energy Production and Biosynthesis by Oral Bacteria

The metabolic pathway by which most oral microorganisms generate the energy (ATP) necessary to support their biosynthetic functions is glycolysis.^{1,2} The glycolytic metabolism of dietary carbohydrates and extracellular or intracellular bacterial polysaccharides yields large amounts of lactic acid since pyruvate, not oxygen, is the terminal electron acceptor in the oxidation of these fermentable energy sources. The anaerobic environment of plaque also makes it obligatory that its microbial components use fermentative mechanisms for energy production. Since most dietary carbohydrates are converted to lactic acid via glycolysis, and since lactic acid production is thought to play a central role in the etiology of dental caries, dietary carbohydrates in addition to sucrose are able to play a causative role in this disease. Likewise, the glycolytic utilization of a broad spectrum of exogenous carbohydrates for energy production and biosynthesis of toxic macromolecules by oral microorganisms also contributes to their role in the etiology of periodontal disease. The relationship of a number of dietary carbohydrates such as sucrose, starch, lactose, maltose, glucose, fructose, mannitol and sorbitol to glycolysis is shown in Figure 2 as is the relationship of bacterial polysaccharide metabolism to this pathway.

Another role of glycolysis in the biosynthetic metabolism of plaque microorganisms, in addition to the generation of energy, is the utilization of certain glycolytic intermediates for macromolecular biosynthesis. The pathways for the incorporation of glycolytic intermediates into macromolecules also enables plaque microorganisms to utilize the carbon dioxide (CO_2) present in saliva for the biosynthesis of cellular constituents. Consequently, the bicarbonate present in saliva may have a nutritional and physiological function for oral microorganisms in addition to being a major contributor of salivary buffering capacity. For instance, *Streptococcus sanguis* converts the glycolytic intermediate

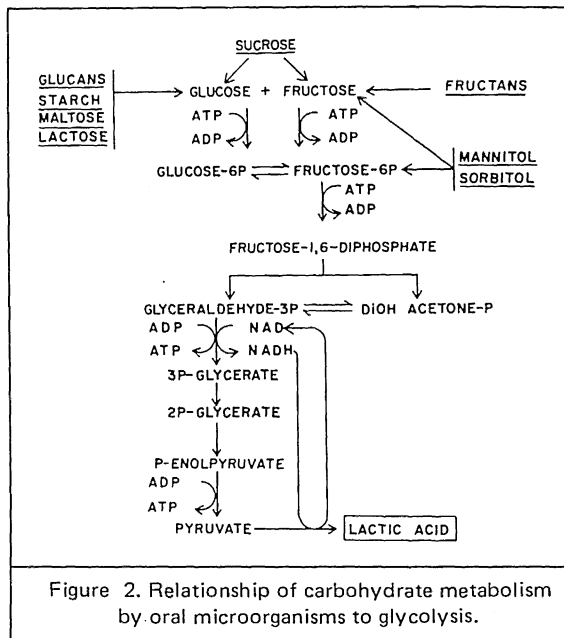


Figure 2. Relationship of carbohydrate metabolism by oral microorganisms to glycolysis.

phosphoenolpyruvate and CO_2 to oxalacetate via phosphoenolpyruvate carboxylase^{1,4} while oral strains of *A. viscosus* and *A. naeslundii* convert pyruvate and CO_2 to oxalacetate via pyruvate carboxylase.^{1,5} The relationship of glycolysis to these reactions is shown in Figure 3. Oxalacetate is most likely incorporated into macromolecules by its conversion to aspartic acid, the role of which is well established in bacterial biosynthetic metabolism.

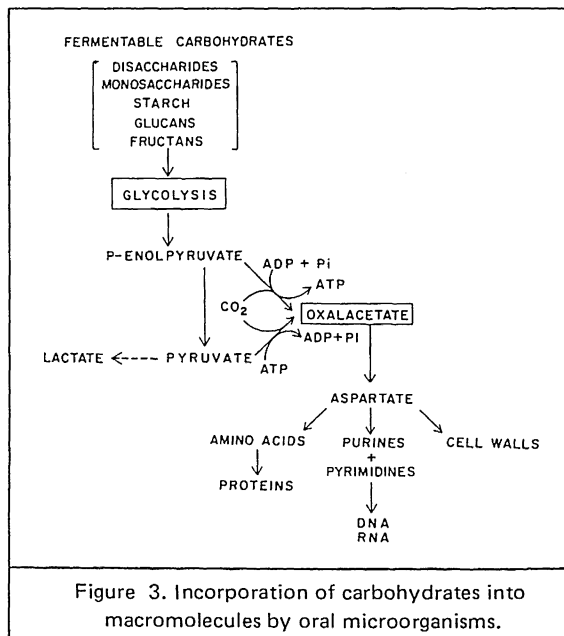


Figure 3. Incorporation of carbohydrates into macromolecules by oral microorganisms.

Summary

It is felt that all dietary carbohydrates, not only sucrose, play a causative role in dental caries and periodontal disease since they collectively contribute to the accumulation of plaque microorganisms on the tooth surface, support the energy-dependent biosynthesis of toxic macromolecules which interact with oral hard and soft tissues, and are precursors of acidic fermentation products which contribute to the demineralization of enamel and cementum.

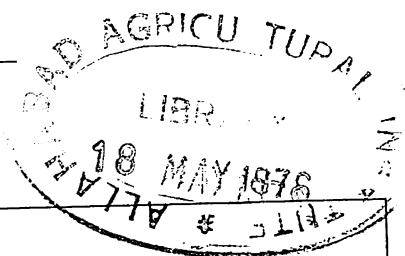
Acknowledgments

The author wishes to express his thanks for the help given by Ms. Stephanie Smith in the preparation of this manuscript and to Dr. Gerald I. Roth for his help and encouragement. □

1. A. Lovdal, A. Arno and J. Waerhaug, *J. Am. Dent. Assn.* 56: 21-33, 1958
2. J.B. MacDonald, R.J. Gibbons and S.S. Socransky, *Ann. N.Y. Acad. Sci.* 85: 467-478, 1960
3. R.J. Gibbons, *Int. Dent. J.* 14: 407-410, 1964
4. H. Loe, E. Theilade and S.B. Jensen, *J. Periodont.* 36: 177-187, 1965
5. A.N. Bahn, *J. Periodont.* 41: 603-610, 1970
6. S.S. Socransky, *J. Dent. Res.* 49: 203-222, 1970
7. A. Tatevossian and G.N. Jenkins, *Arch. Oral Biol.* 14: 1121-1123, 1969
8. I. Kleinberg, *Adv. Oral Biol.* 4: 43-90, 1970
9. K.K. Mäkinen in *Sugars in Nutrition*. H.L. Sipple and K.W. McNutt, Editors, pp. 645-687. Academic Press, New York, 1974
10. T.H. Grenby, *Arch. Oral Biol.* 8: 27-30, 1963
11. B. Guggenheim, K.G. König, E. Herzok and H.R. Mühlemann, *Helv. Odontol. Acta* 10: 101-113, 1966
12. E. Newbrun, *J. Dent. Child.* 36: 239-248, 1969
13. K.G. König and H.R. Mühlemann, *Arch. Oral Biol.* 12: 1297-1298, 1967
14. K.K. Makinen and L. Philosophy, *Int. Dent. J.* 22: 363-386, 1972
15. A.T. Brown in *Sugars in Nutrition*. H.L. Sipple and K.W. McNutt, Editors, pp. 689-719. Academic Press, New York, 1974
16. J. Carlsson, *Odontol. Rev.* (Malmo) 2: 138-160, 1968
17. S. Edwardsson, *Arch. Oral Biol.* 13: 637-646, 1968
18. B. Guggenheim, *Caries Res.* 2: 147-163, 1968
19. R.M. Green and R.L. Hartles, *Arch. Oral Biol.* 14: 235-241, 1969
20. S. Rosen, *Arch. Oral Biol.* 14: 445-450, 1969
21. M.N. Naylor, R.F. Wilson and M.R.B. Melville in *Dental Plaque*. W.D. McHugh, Editor, pp. 41-47. D.C. Thomson and Co., Ltd., Dundee, Scotland, 1970
22. T.H. Grenby, *Arch. Oral Biol.* 16: 631-638, 1971
23. H.L. Ritz, *Arch. Oral Biol.* 12: 1561-1568, 1967
24. J. Carlsson, E. Newbrun and B. Krasse, *Arch. Oral Biol.* 14: 469-478, 1969
25. B. Guggenheim and E. Newbrun, *Helv. Odontol. Acta* 13: 84-97, 1969
26. A.M. Chludzinski, G.R. Germaine and C.F. Schachtele, *J. Bact.* 118: 1-7, 1974
27. H.K. Kuramitsu, *Infection and Immunity* 10: 227-235, 1974
28. H. Mukasa and H.D. Slade, *Infection and Immunity* 10: 1135-1145, 1974
29. R.J. Fitzgerald and P.H. Keyes, *J. Am. Dent. Assn.* 61: 9-19, 1960
30. D.D. Zinner, J.M. Jablon, A.P. Aran and M.S. Saslaw, *Proc. Soc. Exp. Biol. Med.* 118: 766-770, 1965
31. R.J. Gibbons, K.S. Berman, P. Knoettner and B. Kapsimalis, *Arch. Oral Biol.* 11: 549-560, 1966
32. B. Krasse, *Arch. Oral Biol.* 11: 429-436, 1966
33. J.D. de Stoppelaar, J. van Houte and O. Backer Dirks, *Caries Res.* 3: 190-199, 1969
34. N.W. Littleton, S. Kakehashi and R.J. Fitzgerald, *Arch. Oral Biol.* 15: 461-463, 1970
35. J.M. Wood and P. Critchley, *Arch. Oral Biol.* 11: 1039-1042, 1966
36. P. Critchley, J.M. Wood, C.A. Saxton and S.A. Leach, *Caries Res.* 1: 112-129, 1967
37. A. Dahlqvist, B. Krasse, I. Olsson and S. Gardell, *Helv. Odontol. Acta* 11: 15-21, 1967
38. B. Guggenheim and H.E. Schroeder, *Helv. Odontol. Acta* 11: 131-152, 1967
39. R.J. Gibbons and M. Nygaard, *Arch. Oral Biol.* 13: 1249-1262, 1968
40. E. Newbrun, *Caries Res.* 6: 132-147, 1972
41. J.D. de Stoppelaar, K.G. König, A.J.M. Plaschaert and J.S. van der Haven, *Arch. Oral Biol.* 16: 971-975, 1971

42. M.L. Freedman and J.M. Tanzer, *Infection and Immunity* 10: 189-196, 1974
43. H. Mukasa and H.D. Slade, *Infection and Immunity* 8: 555-562, 1973
44. H. Mukasa and H.D. Slade, *Infection and Immunity* 9: 419-429, 1974
44. H. Mukasa and H.D. Slade, *Infection Immunology* 9: 419-429, 1974
45. R.J. Gibbons and R.J. Fitzgerald, *J. Bact.* 98: 341-346, 1969
46. W.A. McDougal, *Aust. Dent. J.* 9: 1-5, 1964
47. R.S. Manly, R. Liberfarb, M. Freese and A. O'Brien, *Int. Assn. Dent. Res. Abstract No.* 201, 1966
48. A. Howell, Jr. and H.V. Jordan, *Arch. Oral Biol.* 12: 571-573, 1967
49. J.M. Wood, *Arch. Oral Biol.* 12: 849-858, 1967
50. J.M. Wood and P. Critchley, *J. Dent. Res.* (Suppl.) 46: 129-130, 1967
51. R.J. Gibbons and S. Banghart, *Arch. Oral Biol.* 13: 297-308, 1968
52. E. Newbrun and S. Baker, *Carbohydrate Res.* 6: 165-170, 1968
53. M. Higuchi, Y. Iwami, T. Yamada and S. Araya, *Arch. Oral Biol.* 15: 563-567, 1970
54. J. Carlsson, *Caries Res.* 4: 97-113, 1970
55. S.M. Garszczynski and J.K. Edwards, *Arch. Oral Biol.* 18: 239-251, 1973
56. H.V. Jordan, P.H. Keyes and S. Lim, *J. Dent. Res.* 48: 824-831, 1969
57. G.J. Hageage, Jr., I. Johanssen and J.M. Tanzer, *Infection and Immunity* 2: 683-685, 1970
58. P.H. Keyes and H.V. Jordan, *Arch. Oral Biol.* 9: 377-400, 1964
59. H.V. Jordan and P.H. Keyes, *Arch. Oral Biol.* 9: 401-414, 1964
60. H.V. Jordan, R.J. Fitzgerald and H.R. Stanley, *Am. J. Path.* 47: 1157-1167, 1965
61. H.V. Jordan, P.H. Keyes and S. Bellack, *J. Period. Res.* 7: 21-28, 1972
62. H.V. Jordan and B.F. Hammond, *Arch. Oral Biol.* 17: 1333-1342, 1972
63. H. Llory, B. Guillo and R.M. Frank, *Helv. Odontol. Acta* 15: 134-138, 1971
64. R.M. Frank B. Guillo and H. Llory, *Arch. Oral Biol.* 17: 1249-1253, 1972
65. B. Guillo, J.P. Klein and R.M. Frank, *Helv. Odontol. Acta* 17: 27-30, 1973
66. B. Rosan and B.F. Hammond *Infection and Immunity* 10: 304-308, 1974
67. J.M. Tanzer W.I. Wood and M.I. Krich-evsky, *J. Gen. Microbiol.* 58: 125-133, 1969
68. C.A. Saxton and P. Critchley in *Dental Plaque*. W.D. McHugh, Editor, pp. 109-127. D.C. Thomson and Co., Ltd., Dundee, Scotland, 1970
69. H.V. Jordan, *Ann. N.Y. Acad. Sci.* 131: 905-912, 1965
70. D.B. Drucker and T.H. Melville, *Arch. Oral Biol.* 13: 563-570, 1968
71. J.M. Tanzer, M.I. Kritchevsky and P.H. Keyes, *Caries Res.* 3: 167-177, 1969
72. D.A. Geddes, *Arch. Oral Biol.* 17: 537-545, 1972
73. G. Charlton, R.J. Fitzgerald and P.H. Keyes, *Arch. Oral Biol.* 16: 649-654, 1971
74. G. Charlton, D.B. Fitzgerald and P.H. Keyes, *Arch. Oral Biol.* 16: 655-661, 1971
75. G.W. Burnett and H.W. Scherp in *Oral Microbiology and Infectious Disease*. Third edition. Williams and Wilkins Co., Baltimore, 1968
76. S.E. Mergenhagen, *J. Dent. Res.* (Suppl.) 51: 251-256, 1972
77. S.E. Mergenhagen, *J. Dent. Res.* 46: 46-52, 1967
78. T.R. Tempel, R. Snyderman, H.V. Jordan and S.E. Mergenhagen, *J. Periodont.* 41: 71-80, 1970
79. S.E. Mergenhagen, T.R. Tempel and R. Snyderman, *J. Dent. Res.* (Suppl.) 49: 256-261, 1970
80. B.F. Hammond, *Arch. Oral Biol.* 14: 879-890, 1969
81. R.J. Gibbons and S.B. Banghart, *Arch. Oral Biol.* 12: 11-24, 1967
82. R.S. Manly, J.H. Kerrigan and I.R. Sklair, *Int. Assn. Dent. Res. Abstract* 505, 1971
83. G.J. Walker, *J. Dent. Res.* (Suppl.) 51: 409-414, 1972
84. K.K. Mäkinen and I.K. Paunio, *Ann. Biochem.* 39: 202-207, 1971
85. R.H. Staat, T.H. Gawronski and C.F. Schachtele, *Infection and Immunity* 8: 1009-1016, 1973
86. R.H. Staat and C.F. Schachtele, *J. Dent. Res. Int. Assn. Dent. Res. Abstract* 53: 211, 1974
87. S.A. Leach in *Dental Plaque*. W.D. McHugh, Editor, pp. 143-156. D. C. Thomson and Co., Ltd., Dundee, Scotland, 1970
88. T. da Costa and R.J. Gibbons, *Arch. Oral Biol.* 13: 609-617, 1968
89. R.S. Manley and D.T. Richardson, *J. Dent. Res.* 47: 1080-1086, 1968
90. J.H. van Houte and H.M. Jansen, *Arch. Oral Biol.* 13: 827-830, 1968

91. R.B. Parker and H.R. Creamer, *Arch. Oral Biol.* 16: 855-862, 1971
92. C.H. Miller, *Infection and Immunity* 10: 1280-1291, 1974
93. H.L. Ritz in *Dental Plaque*. W.D. McHugh, Editor, pp. 17-26. D.C. Thomson and Co., Ltd., Dundee, Scotland, 1970
94. J. van Houte, *Arch. Oral Biol.* 9: 91-93, 1964
95. K.S. Berman and R.J. Gibbons, *Arch. Oral Biol.* 11: 533-542, 1966
96. J.H. van Houte, O. Backer Dirks, J.P. de Stoppelaar and H.M. Jansen, *Caries Res.* 3: 178-184, 1969
97. J. van Houte, C.E. de Moor and H.M. Jansen, *Arch. Oral Biol.* 15: 263-266, 1970
98. J. van Houte and C.A. Saxton, *Caries Res.* 5: 30-43, 1971
99. B.F. Hammond, *Arch. Oral Biol.* 16: 323-338, 1971
100. R.J. Gibbons, *J. Bact.* 87: 1512-1520, 1964
101. K.S. Berman, R.J. Gibbons and J. Nalbandian, *Arch. Oral Biol.* 12: 1133-1138, 1967
102. J.R. DiPersio, S.J. Mattingly, M.L. Higgins and G.D. Shockman, *Infection and Immunity* 10: 597-604, 1974
103. H. Nikaido and W.Z. Hassid, *Advances Carbohydrate Chem.* 26: 351-483, 1971
104. T. Yamada and J. Carlsson, *Arch. Oral Biol.* 18: 799-812, 1973
105. A.T. Brown, unpublished observation



Nutrition Reviews

authoritative articles by distinguished international scientists • competent, critical, and knowledgeable reviews • interpretative summaries of important original clinical and experimental research • selected excerpts from classic publications marking milestones in nutrition research • informative special reports on government and organization actions • contributing editors review journals of widespread interest covering biochemistry, food science, public health, toxicology, dentistry, pediatrics, internal medicine, gastroenterology and other clinical specialities •

Send subscription and renewal requests to:

THE NUTRITION FOUNDATION
888 Seventeenth Street, N.W.
Washington, D. C. 20006

\$12.00 per year

\$6.00 for students

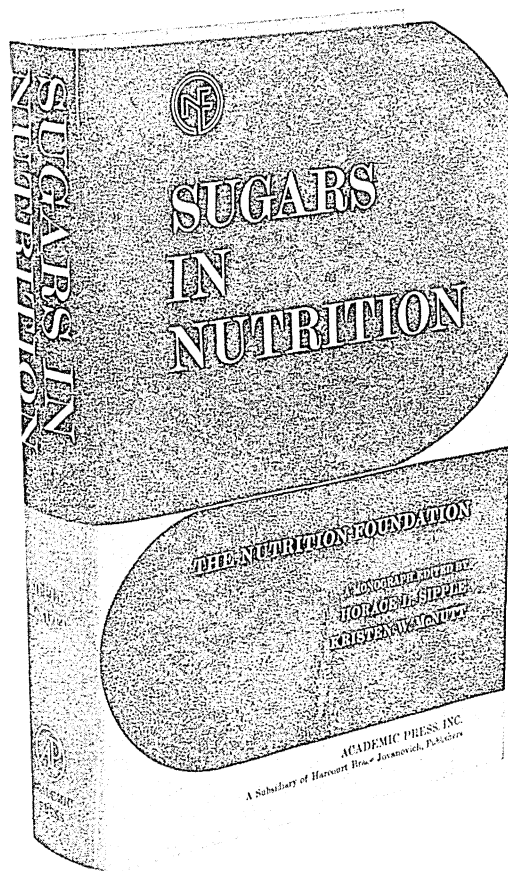
New from Academic Press

SUGARS IN NUTRITION

A Nutrition Foundation Monograph

Presents the most recent research findings on the role of sugars in the nutrition of man. The contributors range from biochemists and clinical chemists to dentists, economists, food scientists, historians, internists, medical researchers, nephrologists, nutritionists, ophthalmologists, pediatricians, pharmacologists and physiologists.

776 pages, \$45.00 (£21.60) Order from:
Academic Press, 111 Fifth Avenue,
New York, New York 10003
or your book supplier.



CONTENTS

State of the Art, Past and Present

- Sugar in History
- Present Knowledge of Sugars in Nutrition—An Overview

Biochemistry and Psychology of Sweetness

- Biochemistry of Sweet Sensation
- The Psychology of Sweetness

Sugars in Food: Occurrence and Usage

- Occurrence of Various Sugars in Foods
- World Sugar Production and Usage in Europe
- Level of Use of Sugars in the United States

Sugars in Food: Recent Technological Developments

- The Chemistry and Technology of Sugars
- Current Developments in Industrial Uses for Sugars
- New Carbohydrate Sweeteners

Digestion and Absorption of Sugars

- Hydrolysis and Absorption of Carbohydrate, and Adaptive Responses of the Jejunum
- Adaptive Effects of Dietary Sugars on Intestinal Disaccharidase Activity in Man
- Enzyme Deficiency and Malabsorption of Carbohydrates
- Effect of Carbohydrates on Intestinal Flora

Metabolism of Sugars

- The Metabolism of Polyols
- The Metabolism of Xylitol
- Comparative Metabolism of Xylitol, Sorbitol, and Fructose
- Metabolism of Lactose and Galactose

- The Effects on Metabolism of Maltose and Higher Saccharides
- Involvement of the Raffinose Family of Oligosaccharides in Flatulence

Disorders Related to Sugar Metabolism

- Inborn Errors of Carbohydrate Metabolism
- The Metabolism and Toxic Effects of Fructose
- Cataractogenic Effects of Lactose and Galactose
- Obesity
- Sugars in Cardiovascular Disease
- Influence of Dietary Fructose and Sucrose on Serum Triglycerides in Hypertriglyceridemia and Diabetes
- Pediatric Diabetes
- The Sorbitol Pathway in the Nervous System
- Experimental Models of Diabetes
- Adult Diabetes

Therapeutic Use of Sugars

- Sugars in Parenteral Nutrition
- Toxicity of Parenteral Xylitol
- The Safety of Oral Xylitol
- The Renal Metabolism and Uses of Mannitol
- Xylitol as a Therapeutic Agent in Glucose-6-Phosphate Dehydrogenase Deficiency

Sugars in the Oral Cavity

- Carbohydrates as a Causative Factor in Dental Caries: Epidemiological Evidence
- Sugars and the Formation of Dental Plaque
- Carbohydrate Metabolism in Caries-Conducive, Oral Streptococci

New from Academic Press

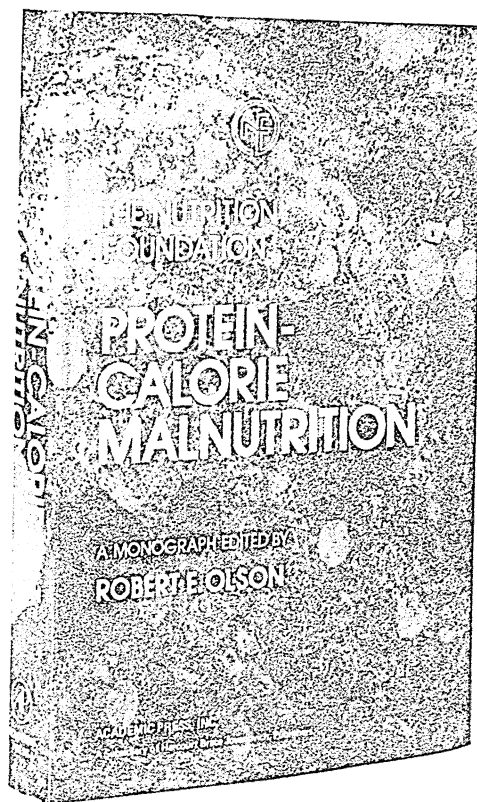
PROTEIN-CALORIE MALNUTRITION

A Nutrition Foundation Monograph

For physicians, nutritionists, public health practitioners, social workers, and medical students.

Covers all aspects of protein-calorie malnutrition (PCM), from molecular biology to epidemiology and prevention.

467 pages, \$29.50. Order from:
Academic Press, 111 Fifth Avenue,
New York, New York 10003
or your book supplier.



CONTENTS

Amino Acid Requirements and Age

Adaptation to Low-Protein Intakes

Protein Turnover in Various Nutritional States

Lysine Metabolism in Protein-Calorie Malnutrition with
Attention to the Synthesis of Carnitine

Cellular Energy Metabolism in Protein-Calorie Malnutrition

Brain DNA Synthesis in Protein-Calorie Malnutrition

Electroencephalograms, Learning, and Malnutrition

The Relationship of Severe Marasmic Protein-Energy
Malnutrition and Rehabilitation in Infancy to Subse-
quent Mental Development

Carbohydrate and Lipid Metabolism in Protein-Calorie
Malnutrition

Water-Soluble Vitamins in Protein-Calorie Malnutrition

Nutritional Survey of Children in the Dominican Republic
with Attention to Deficits in Water-Soluble Vitamins

The Fat-Soluble Vitamins and Protein-Calorie (Energy)
Malnutrition

Mineral Metabolism in Protein-Calorie Malnutrition

Mineral Metabolism in Protein Malnutrition

Endocrines and Malnutrition

Erythropoiesis in Protein-Calorie Malnutrition

The Anemia of Protein-Calorie Malnutrition

The Effect of Vitamin E on the Anemia of Protein-Calorie
Malnutrition in Northern Thai Children

The Effect of Variations in Protein and Calorie Intake on
the Rate of Recovery and Selected Physiological Re-
sponses in Thai Children with Protein-Calorie
Malnutrition

The Liver in Protein-Calorie Malnutrition: An Ultrastruc-
tural Study

Calories in the Treatment of Protein-Calorie Malnutrition

Protein-Sparing Effect of Excess Calories during Adapta-
tion to Low-Protein Intake

Protein versus Calories in the Treatment of Protein-Calorie
Malnutrition: Metabolic and Population Studies in India

Interactions of Malnutrition and Infection: Advances in
Understanding

Immunoglobulins and Complement in Protein-Calorie
Malnutrition

Cell-Mediated Immunity in Protein-Calorie Malnutrition

Treatment of Severe Protein-Calorie Malnutrition

The In-Patient and Out-Patient Treatment of the Child
with Severe Protein-Calorie Malnutrition

Prevention of Vitamin and Mineral Deficiencies Associated
with Protein-Calorie Malnutrition

Prevention of Protein-Calorie Malnutrition

AUTHOR INDEX

The letters SA preceding the page number designate authors of special signed articles; the letter N indicates authors mentioned in the Notes section; LE refers to authors of Letters to the Editor; and B designates authors or editors of recent books listed in the Notes section.

Names beginning Mc are indexed before those beginning with Ma.

- | | | |
|--------------------------|----------------------------|------------------------------------|
| Abaci, F., 271 | Arnold, R.M., 5 | Beare-Rogers, J.L., 167 |
| Abd-El-Hadi, K., 134 | Aronson, A.E., 53 | Beas, F., 91, 302 |
| Abeles, R.H., 87, 219 | Arvidson, G., 205 | Beaton, G.H., 216, 296 |
| Abrams, G.D., 325 | Arvidsson, B., 240 | Becker, D.E., 68 |
| Abuid, J., 302 | Arvidsson, V.B., 43 | Becker, F.F., B191 |
| Achuta Murthy, P.N., 101 | Astwood, E.B., 172 | Becker, J.E., 48 |
| Adam, D.J.D., 331 | Attebery, H.K., 326 | Beeler, D.A., 204, 205 |
| Adams, W.H., 37 | Auld, P., 87 | Begum, A., 165 |
| Addiego, J.E., 14 | Avioli, L.V., 210 | Behne, D., 134 |
| Addison, G.M., 14 | Aykroyd, W., 128 | Behrle, F.C., 299 |
| Admirand, W.H., 204 | Aykroyd, W.R., 233 | Beisel, W.R., 134 |
| Aftergood, L., 166 | Aylward, F., B351 | Belavady, B., 43, 296 |
| Ahrens, E.H., Jr., 238 | Azzam, S.A., 165 | Bell, S., 74 |
| Åkesson, B., 205, B319 | | Bellack, S., 360 |
| Alavione, R., 238 | Babior, B.M., B320 | Belt, T.A., 260 |
| Albanese, A.A., 202 | Backer Dirks, O., 359, 361 | Bender, A.E., 126 |
| Albrink, M.J., 197 | Baens, G.S., 169 | Benjamin, I.T., 334 |
| Albutt, E.C., 198 | Baertl, J.M., 91 | Benkovic, S.J., 101 |
| Alexander, C.S., 69 | Baggenstoss, A.H., 40 | Bergeim, O., 325 |
| Alfaro, B., 309 | Bahn, A.N., 359 | Berggård, I., 245 |
| Alfin-Slater, R.B., 166 | Bain, J.A., 216 | Bergstrom, J., 297 |
| Alhers, H., 296 | Baird, J.D., 197 | Berman, K.S., 359, 361 |
| Allaway, W.H., B256 | Baker, H., 299 | Berman, W., N223 |
| Alleyne, G.A.O., 76, 302 | Baker, S., 360 | Berneske, G.M., 270 |
| Alling, C., 281 | Baker, S.J., 165, 334 | Bernhard, K., 166 |
| Almeida, J.D., N287 | Balassa, J.J., 140 | Bernsohn, J., 281 |
| Alter, M., 189 | Balchum, O.J., 166 | Berry, W.R., 53 |
| Alvarado, J., 76 | Balint, J.A., 204, 205 | Beutler, E., 214 |
| Amine, E.K., 47, 56 | Ball, E.G., 343 | Bhagavan, H.N., 22, 23 |
| Ammann, A.J., 337 | Banghart, S., 360 | Bhuyan, B.K., 117 |
| Anast, C.S., N223 | Banghart, S.B., 360 | Bianchi, A., 37 |
| Andelman, M.B., 145 | Banwell, J.G., N287 | Bibby, B.G., 140 |
| Andersen, A.E., 277 | Bar, A., 5 | Bickel, L., 128 |
| Anderson, A.J.B., 326 | Barbieri, D., 110 | Bieber, C.P., 14 |
| Anderson, C.E., 198 | Barley, F.W., 219 | Biely, J., 166, 270 |
| Anderson, C.F., B256 | Barnes, J.M., 20 | Biemann, K., 219 |
| Andrews, E.D., 68 | Barnes, L.L., 241 | Bieri, J.G., SA 161, 165, 166, 167 |
| Antar, M.A., 45 | Barnes, P.M., 329 | Bierman, E.L., 197 |
| Antonioli, J.A., 284 | Barness, L.A., 165 | Binder, H.J., 165 |
| Aperia, A., 107 | Barnett, H.L., 62 | Birdwell, B.J., 69 |
| Arakawa, T., 22, 23, 58 | Baron, D.N., 296 | Birge, S.J., 210 |
| Aran, A.P., 359 | Barron, G.P., 56 | Bishop, R.F., N287 |
| Aranda, J.V., 169 | Barros, N.G., 110 | Björn-Rasmussen, E., 214, 240 |
| Araya, S., 360 | Bassiri, R.M., 91 | Blackmore, D., 91 |
| Archibald, J.C., 260 | Basu, D., 43 | Blakley, R.L., 120 |
| Archibald, J.G., 68 | Baugh, C.M., 117 | Blanc, W.A., 111 |
| Arees, V., 137 | Baum, H., 166 | Blanton, T., 210 |
| Arenas, S., 302 | Bauman, H.E., B31 | Bledsoe, T., 302 |
| Arguedas G., J.A., 332 | Bauman, L.F., B191 | Blizzard, R.M., 91, 302 |
| Arias, I.M., 217 | Bayless, T.M., 36 | Blokhima, R.I., 69 |
| Arky, R.A., 197 | Bazin, R., 188 | Blomstrand, R., 166 |
| Arno, A., 359 | Beamish, M.R., 14 | Blunt, M.H., 145 |

- Boddington, M.M., 36
 Boggs, T.R., 56
 Boisseau, V.C., 210
 Bond, J.H., Jr., 326
 Booyens, J., 197
 Bordier, P., 210
 Borgstedt, A.D., N223
 Borgström, B., B319
 Bornside, G.H., 105
 Bortz, W.M., 101
 Bouchard, B.S., 296
 Bouis, P.J., Jr., 105
 Bourne, G.H., B32, B319
 Bowen, W.H., 140
 Boyer, J., 197
 Bradfield, R., 128
 Brady, R.N., 101, 102
 Braithwaite, D.M., 45
 Brandone, A., 135
 Brans, Y.W., 9
 Braverman, E., 117
 Bremer, H.J., 329
 Brenner, B.M., 107
 Brereton-Stiles, G.G., 111, 336
 Bressler, R., 346
 Brewer, G.J., 267
 Bridgeforth, E.B., 334
 Bridgeforth, E., 296
 Briggs, W., 210
 Brill, A.B., 80
 Brin, M., 22, 216
 Brink, N.G., 68
 Briquet, M.C.M., 110
 Broberger, O., 107
 Brokken, T.D., 5
 Bross, I., 326
 Brown, A.T., SA353, 359, 361
 Brown, F.A., 172
 Brown, G.M., 217
 Brown, H., 260
 Brown, M.L., B320
 Brown, R.R., 296
 Brozek, J., 23
 Bruce, A., 281
 Bruice, T.C., 101
 Brumbaugh, P.F., 5
 Brunner, H., 171
 Brunström, G.M., 14
 Bryden, A.S., N287
 Bryson, M.F., N223
 Buchanan, O.H., 326
 Buchley, R.D., 166
 Buehring, K.U., 117
 Bullen, J.J., 105
 Bunnell, R.H., 167
 Burch, R.E., 69
 Burger, W.F., 36
 Burkeholder, J.N., 134
 Burkitt, D.P., 72, 137, 326
 Burko, H., 80
 Burnett, G.W., 360
 Burt, R.L., 134, 135
 Burton, E., 117
 Buselmeir, T.J., N288
 Bushek, W., N288
 Butson, A.R., 270
 Butz, E.L., 234
 Caasi, P.I., 166
 Caddell, J.L., 76
 Caffyn, Z.E., 25
 Cahill, G.F., 302
 Callendar, S.T., 36
 Cama, H.R., 43
 Camberos, H.R., 5
 Campbell, G.D., 197
 Campbell, J.A.H.S., 337
 Campbell, P.A., 166
 Campbell, P.E., 189
 Campos, C.M., 5
 Campos, J.L.E., 5
 Cannon, R.O., 296
 Canter, A.H., 166
 Carballo, A., B191
 Cardenas, R., 166
 Cardi, L., 229
 Cardinale, G., 219
 Cardinale, G.J., 87
 Carey, R.E., 346
 Carlisle, E.M., SA257, 260, 261
 Carlson, L.A., 297
 Carlsson, J., 359, 360, 361
 Carlton, W.W., 308
 Carmel, R., 36
 Carnes, W.H., 309
 Caroline, L., 105
 Carpenter, M.P., 166
 Carr, J.G., B351
 Carrillo, B.J., 4, 5
 Carswell, F., 268
 Carter, J.P., 134
 Cartwright, E., 189
 Cartwright, G.E., 14, 214, 309
 Carty, T.J., 219
 Cassidy, G., 8, 107
 Cassidy, C.E., 172
 Castelli, W.P., N286
 Catignani, G.L., Jr., 60, 166
 Catovsky, D., 111
 Cavallo, A., 110
 Chadd, M.A., 165
 Chalmers, T.M., 210
 Chaloupka, M.M., 296
 Chan, H., 182
 Chanarin, I., 120
 Chance, G.W., 198
 Chandra, R.K., 105, 111, 336, 337
 Charlton, G., 360
 Charnot, Y., 260
 Chatterjee, B.D., N287
 Cheek, D.B., 94, 302
 Chen, S.C., 261
 Cheney, M.C., 216
 Chhetri, M.K., 302
 Chiba, F., 22, 23
 Chichester, C.O., 126
 Childers, G., 208
 Chipman, D.W., 37
 Chludzinski, A.M., 359
 Chopek, M., 40
 Chopra, I.J., 302
 Chow, C.K., 166
 Christakis, G., B32
 Christensen, H.N., 284
 Christian, W., 296
 Churchich, J.E., 217
 Chytil, F., 60, 166
 Claeyssen, G., 91
 Clark, J.N., 245
 Classick, L.G., N287
 Cleave, T.L., 197
 Cloutier, R.J., 25
 Coggeshall, L.T., N317
 Cohen, A.M., 197
 Cohen, B.E., 166
 Cohen, G., 166
 Cohen, H.J., 186
 Cohen, I.K., 166
 Cohen, P.A., 216
 Cohn, I., Jr., 105
 Cole, G., 219
 Coles, B.L., 68
 Colle, E., 169
 Collins, M.E., 198
 Comar, C.L., 68
 Conn, H.F., 198
 Connor, W.C., 74
 Connor, W.E., 197, 198, 331
 Conrads, M.E., 68
 Consolazio, C.F., 69
 Contreras, I., 302
 Conway, M., 200
 Cook, J.D., 14, 214
 Cook, J.W., 137
 Cook, M.J., 68
 Cooper, H.R., 166
 Cooper, H.S., 190
 Cooper, T.G., 101
 Coovadia, H.M., 111, 336
 Corcino, J.J., 36
 Cordano, A., 302
 Cornatzer, W.E., 134, 135
 Cornblath, M., 169, 271
 Corner, G.W., N317
 Corradino, R.A., 5
 Corrigan, J.J., Jr., 270
 Corrocher, R., 117
 Coulson, R.A., 284
 Coulson, W.F., 309
 Coursin, D.B., 22, 23, 198
 Coward, W.A., 337
 Cox, E.V., 87, 219
 Craddock, C.G., 111
 Craig, P.H., 5
 Cravioto, J., B32
 Crawford, M.A., 281

- Creamer, H.R., 361
 Creighton, C., 296
 Critchley, P., 359, 360
 Crosby, N.T., 20
 Crosby, W.H., 69
 Cross, E.R., 145
 Cummings, J.H., 72, 325
 Cummings, M.M., N317
 Cushnie, G.H., 105
 Cuthbert, J.E., 166
 Cutler, J.L., 296
 Cutting, C.V., B351
- da Costa, M., 11, 184
 da Costa, T., 360
 Dahlqvist, A., 359
 Dahm, C.H., Jr., 166
 Dakshinamurti, K., 22, 23
 Dalakos, T.G., 135
 Dallman, P.R., 14, 208
 Dämmrich, K., 5
 Daniel, P., 5
 Danks, D.M., 189
 Dann, W.J., 296
 Darby, W.J., 47, 60, 165, 166,
 SA225, 234, SA289, 296
 Darley, F.L., 53
 Das, T.K., 182
 Daughaday, W.H., 265
 Daugharty, T.M., 107
 Dauncey, M.J., 238
 Davi, E., 172
 Davidson, G.P., N287
 Davidson, I.W.F., 134, 135
 Davidson, J.K., 198
 Davie, M.W., 210
 Davies, H., N287
 Davies, J.N.P., 297
 Davies, T., 166
 Davis, G.K., 5, 68
 Davis, J.T., 134
 Davison, A.N., 7
 Dawson, D.W., 36
 Day, E.A., 47
 Dayrell, M.D., 5
 Dayton, S., 74, 197
 Dean, R.F.A., 297
 Deb, A.K., 43
 Debons, A.F., 25
 DeCarli, L.M., 80
 DeCosta, J.B.D., 5
 De Haas, J.H., 191
 Delaney, R., 28
 de Lange, D.J., 297
 Delivoria-Papadopoulos, M., 145
 DeLuca, H.F., 5, 167
 DeMartinis, F.D., 281
 DeMayer, E.M., 296
 de Moor, C.E., 361
 Dempsey, M.E., 205
 DenBesten, L., 74, 331
 Densen, P.M., 296
- Dent, C.E., N223, 296
 Deodhar, A.D., 43
 Derby, M.B., 296
 Deshpande, P.D., 5
 de Stoppelaar, J.D., 359, 361
 de V. Frank, M., 197
 De V. Heese, H., 271
 de Waal, V.W., 197
 Dhar, A.K., 205
 Dhopeshwarker, G.A., 281
 Dickson, E.R., 40
 Diehl, F., 134
 Diener, M.M., 111
 Dietz, A.A., 166
 Dimroth, P., 101
 Dinning, J.S., 60, 165, 166
 DiPaolo, R., 189
 DiPersio, J.R., 361
 Diplock, A.T., 166
 Dirksen, G., 5
 di Sant'Agnese, P., 165
 Djafor, M.I., 5
 Dobbins, J., 7, 94
 Döbereiner, J., 5
 Dodge, J.T., 166
 Doisy, R.J., 134, 135
 Dolby, A.E., 105
 Donaldson, B., 20
 Donati, R.M., 146
 Donner, A., N286
 Dormandy, T.L., 166
 Dowdy, R.P., B31, 297
 Dragstedt, L.R., N317
 Draper, W.L., 43
 Draser, B.S., 137
 Dreskin, A., 37
 Drew, R.T., 186
 Dreyfus, P.M., 87
 Drucker, D.B., 360
 Drummond, J.C., 295
 Dubach, R., 240
 Dubowitz, L.M.S., 169
 Dubowitz, V., 169
 Ducluzeau, R., 325
 Dudrick, S.J., 271
 Dulin, W.E., 197
 DuPlessis, J.P., 297
 Dupont, H.L., N287
 Durnin, J.G.V.A., 325
 Dussault, J.H., 94
 Dweck, H.S., 8
 Dyck, P.J., N288
 Dymsha, H.A., 270
- Eastwood, M.A., 72, 325, 326
 Eaton, H.D., 110
 Eaton, R.P., 200
 Ebel, J.G., 5
 Ebner, K.E., B320
 Edelmann, C.M., Jr., 107
 Edgar, P.J., 302
 Edkins, S.M., 198
- Edwards, J.R., 360
 Edwards, K.D.G., 8
 Edwardsson, S., 359
 Egan, W., 28
 Eggerer, H., 87
 Eisenberg, M.A., 101
 Ekelund, L.G., 297
 Eklung, M.W., 122
 El Cholmi, A., 134
 Eldridge, F.L., 42
 Ellis, G.H., 68
 el Lozy, M., 43
 Elvehjem, C.A., 295, 296
 Elwood, P.C., 56, 334
 Elwyn, D.H., 284
 Embree, N.D., 167
 Emerson, P.M., 166
 Emtage, J.S., 5
 Enders, J.F., N317
 Engel, G.L., N317
 Engel, R.W., 68
 Engman, M.F., Jr., 261
 Engman, M.F., Sr., 261
 Engsner, G., 109
 Engström, I., 109
 Enser, M., 216
 Epstein, J., 167
 Epstein, S.S., 20
 Erdody, P., 140, 167
 Erenberg, A., 94
 Eriksson, S., 190
 Ershoff, B.H., 326
 Erslev, A.J., 214
 Evans, G.W., 135
 Evans, H.M., 265
 Evans, R.L., N288
- Faherty, T.P., 110
 Fales, H.M., 277
 Fang, M., 55
 Farell, P.M., 270
 Faroozan, P., 36
 Farquhar, J.W., 200, 238
 Feinblatt, J., 261
 Feinendegen, L.E., 329
 Feinstone, S.M., 332
 Feldman, F.J., 134
 Felig, P., 302
 Fenner, H., 260
 Fenselau, C., 101
 Ferguson, A., 268
 Ferguson, A.C., 111
 Ferguson, M.E., 296
 Fernelius, A.L., N287
 Fernlund, P., 28
 Ferreira, B.W.I., 110
 Ferrer, J.M., B32
 Fielding, J., 14
 Filipsson, R., 265
 Filmer, J.F., 68
 Finch, C.A., 14, 214
 Finch, S., 14

Finchem, I.H., 5
 Findlay, J.M., 326
 Finegold, S.M., 326
 Firschein, H.E., 260
 Fischer, E.H., 217
 Fiser, R.H., Jr., 169
 Fish, M.B., 165
 Fisher, D.A., 94
 Fisher, E.A., 182
 Fisher, J.W., 69
 Fisher, N., 325
 Fitch, D.M., 69
 Fitzgerald, D.B., 360
 Fitzgerald, R.J., 359, 360
 Flavin, M., 87
 Fleck, T.R.C., 343
 Fletcher, D.C., B160
 Fletcher, J., 105
 Flewett, T.H., N287
 Florkin, M., 101
 Folin, O., 141
 Folkers, K., 68
 Fomon, S.J., B32
 Forbes, A.E., 271
 Ford, J.E., 184
 Fordtran, J.S., 268, 326
 Foreman, J.K., 20
 Formal, S.B., N287
 Forsgren, L., 166
 Forshaw, J., 37
 Forsythe, J.H., 238
 Forth, W., 68
 Foster, D.W., 349
 Fouts, P.J., 295
 Frank, O., 299
 Frank, R.M., 360
 Frankston, J.E., 202
 Fraser, A.J., 165
 Fredrickson, D.S., 175, 296
 Freedman, M.L., 360
 Freese, M., 360
 Frenkel, E.P., 87
 Frenkel, R., 87
 Fried, W., 69
 Friedman, H., 332
 Friedman, M., B319
 Frisancho, A.R., 8
 Frisch, B., 111
 Fritz, H.P., 87
 From, A., 25
 Frood, J.D.L., 337
 Froozani, M., 56
 Frost, D.V., 140
 Fullmer, C.S., 5
 Funck-Brentano, J-L., N288
 Funk, C., 176, 295
 Funk, I.C., 295

Gabrieli, E.R., 105
 Gaggino, O.P., 5
 Gairdner, D.G., 145
 Gairdner, D.M.T., 238

Gaitan, E., 172
 Gallagher, M.I., 146
 Galli, C., 281
 Galloway, R.E., 267
 Galton, D.A., 111
 Ganther, H.E., 134, 166
 Gardell, S., 359
 Gardner, F.H., 68
 Garn, S.M., 8
 Garrison, M.M., 343
 Garrod, A.E., 81
 Garrow, J.S., 76
 Garsczynski, S.M., 360
 Gatmaitan, Z., 217
 Gauld, E.N., 270
 Gaull, G.E., 216
 Gawronski, T.H., 360
 Gawthorne, J.M., 120
 Gazal, S., 110
 Gebauer, P.W., 5
 Geddes, D.A., 360
 Gellene, R., 299
 Genest, J., N317
 George, J.N., 69
 Germaine, G.R., 359
 Gerritsen, G.C., 197
 Gershon, D., 167
 Ghadimi, H., B191, 271
 Ghitis, J., 184
 Gibbens, J., 296, 303
 Gibbons, J., 359, 360, 361
 Gibson, D.M., 101
 Gibson, N., 296
 Gilbert, J.J., 167
 Gilman, A., 270
 Gilman, A.G., 58
 Gilson, A.J., 36
 Ginsberg-Fellner, F., 216
 Girardot, J-M., 28
 Glaser, E., 134
 Gleiss, J., 8
 Glew, R.H., 190
 Glick, Z., 25
 Glinsmann, W.H., 134
 Glista, B., 299
 Gloor, U., 166
 Go, V.L.W., 171, 204, N285
 Goddard, D.R., 76
 Goettlich-Riemann, W., 308
 Gold, H., 326
 Goldberg, M.D., 197
 Goldbert, C., 169
 Goldbourt, V., 197
 Goldman, C., 165
 Goldman, J.M., 111
 Goldsmith, G.A., 296, 303, N317
 Goldstein, B.D., 166
 Goldstein, E., 105
 Goldstein, F., 36
 Goldstein, N.P., 53
 Goldwasser, E., 69
 Gontzea, I., B32

Goodman, D.S., 245
 Goodman, L.S., 270
 Goodridge, A.G., 343
 Gopalan, C., 43, 296
 Gorbach, S.L., N287
 Gorby, W., 347
 Gorby, W.G., 347
 Gordon, H.H., 165
 Gordon, J.E., 7, 336
 Gordon, M.H., 165
 Gorten, M.K., 145
 Gougas, E., 172
 Gough, K.R., 62
 Grace, H.J., 111, 336
 Graham, G.G., 91, 302
 Grant, W.C., 68
 Green, H.L., 58
 Green, J., 166
 Green, J.A., Jr., 186
 Green, R., 219
 Green, R.M., 359
 Green, S.G., 80
 Greenaard, J., 271
 Greenberg, A.H., 91
 Greene, F.E., 135
 Greene, L.H., 58
 Greenwald, I., 172
 Greenwood, C.T., 325
 Gregory, D.H., 205
 Greiner, T., B256, 326
 Grenby, T.H., 359
 Griesser, R., 101
 Grimaldi, R.D., 238
 Grinvalsky, H.T., 69
 Grisolia, S., 101
 Gross, S., 166, 208
 Grossman, M., 165
 Guchhait, R.B., 101
 Gueri, M.C., 267
 Guest, J.R., 117
 Guggenheim, B., 359
 Guillo, B., 360
 Gulgasenko, A.I., 140
 Gunther, M., 43
 Gupta, K., 334
 Gurney, M.W., 182
 Guroff, G., 277
 Gürson, C.T., 134, 135
 Guthrie, H.A., 56

Habib, Y.A., 43
 Habif, D.V., B32
 Habte, D., 109
 Hackman, A.S., 137
 Haddad, J.G., 210
 Hadjimarkos, D.M., 140
 Hadley, J.G., 166
 Hafeman, D.G., 166
 Haffron, D., 296
 Hageage, G.J., Jr., 360
 Hagler, L., 58, 329
 Hahn, P., 169

- Hahn, T.J., 210, N223
 Haines, W.J., 202
 Hales, C.N., 14
 Hall, G.A., 309
 Hall, K., 265
 Halberg, L., 214, 240
 Halpern, G., 134
 Halsted, C.H., SA33, 37
 Halvorsen, K., 146
 Halvorsen, S., 146
 Hambidge, K.M., 134, 329
 Hambraeus, L., B32
 Hamilton, D., 325
 Hamilton, H.G., 69
 Hamilton, J.G., 55
 Hamilton, S.R., 190
 Hammick, J.W., 19
 Hammond, B.F., 360, 361
 Hamosh, M., 206, 343
 Hanafy, M.M., 43
 Handreck, K.A., 260
 Hänichen, T., 5
 Hansen, A.E., 331
 Hanson, R.F., 205
 Hara, K., 58
 Harden, M.L., B31
 Harke, H.T., Jr., 111
 Harms, R.H., 5
 Harrar, J.G., 233, 234
 Harris, H., 296
 Harris, J.W., 219
 Harris, L.J., 296
 Harris, P.L., 167
 Harris, R.E., 166
 Harris, R.S., 331
 Harrison, H.E., 62
 Harrison, T.R., N317
 Hart, E.W., 296
 Hartles, R.L., 359
 Harvey, C.C., 166, 296
 Hashim, S.A., 165
 Haskins, D., 14
 Hassan, A., 296
 Hassan, H., 165
 Hasselbach, W., 281
 Hassid, W.Z., 361
 Hastings, A.B., N317
 Hauer, E.C., 134
 Haurani, F., 36
 Hauschild, A.H.W., 122
 Haussler, M.R., 5
 Hauswirth, J.W., 166
 Havel, R.J., 200, 297
 Havlicek, V., 22
 Hawk, P.B., 325
 Hawsworth, G., 137
 Hayashi, T., 58
 Hayes, K.C., 110, 346
 Hazlewood, C.F., 76
 Heaton, F.W., 309
 Heaton, K.W., 325
 Hegarty, A.F., 101
 Hegsted, D.M., 45, 47, 56, 202, 238
 Heinen, W., 260
 Heinzerling, R.H., 166
 Heizer, W.D., 186
 Helmer, O.M., 295
 Hendin, B.A., 210, N223
 Herbert, J., 337
 Herbert, J.D., 284
 Herbert, V., 36, 37, 219
 Herman, J.B., 297
 Herman, R.H., 58, 329
 Herman, Y.F., 58
 Hermann, A.H., 74
 Hermanns, M., 8
 Hermos, J.A., 37
 Hernandez, T., 284
 Herrera, M.G., 198
 Hersh, L.B., 87
 Hershko, C., 214
 Herzog, J., 188
 Herzok, E., 359
 Hesse, R., 210
 Hetem, S., 180
 Higashi, O., 58
 Higgins, E.S., 186
 Higgins, M.L., 361
 Higgs, S.C., 271
 Hignite, C., 219
 Higuchi, M., 360
 Hill, C.H., 308
 Hill, L., 346, 347
 Hill, L.J., 346, 347
 Hill, M.J., 137, 326
 Hillebrand, S.S., 197
 Hilleman, M.R., 332
 Hilsheimer, R., 122
 Himsworth, H.P., 197
 Hines, J.D., 219
 Hirsch, A., 296
 Hirsch, J., 238
 Hodkins, M., 14
 Hoekstra, W.G., 134, 166
 Hoffbrand, A.V., 117
 Hoffman, N.E., 204
 Hofmann, A.F., 171, 204, N285
 Holden, R.J., 268
 Holick, M.F., 5
 Holliday, M., 265
 Hollingsworth, D., 238
 Hollingsworth, D.F., 126
 Hollis, D., 101
 Holman, E., N317
 Holman, R.T., 331
 Holmes, I.H., N287
 Holmgren, A., 297
 Holmgren, H., 105
 Holt, A.B., 94
 Holt, L.E., Jr., 202
 Holzbach, R.T., 80
 Hommes, F.A., 208
 Honda, Y., 23, 58
 Hopkins, F.G., 15
 Hopkins, L.L., Jr., 134
 Hopkins, P.S., 94
 Horecker, B.L., 216
 Hornick, R.B., N287
 Horwitt, M.K., 165, 166, 296
 Horwitz, I., 91
 House, H.L., 167
 Hove, E.L., 166
 Howard, C.N., Jr., 166
 Howard, J.B., 28
 Howell, A., Jr., 360
 Howell, J. McC., 309
 Hoy, W., 40
 Huber, C., 295
 Huddow, A., 137
 Huggins, C.B., N317
 Hughes, R.C., 69
 Huisman, T.H.J., 145
 Hulstaert, C.E., 166, 208
 Hultman, E., 297
 Humphreys, D.J., 5
 Humphreys, S.R., 40
 Hunt, C.E., 308
 Hunt, J.C., B256
 Hunter, J., N223
 Hunter, J.O., 210
 Hussein, M.A., 182
 Hutchinson, J.B., 325
 Huxtable, R., 346
 Iammarino, R.M., 56, 190
 Im, W.B., 102
 Ingold, K.U., 47
 Irby, V., 202
 Irreverre, F., 186
 Irwin, M.I., 202
 Isaksson, B., 240
 Ismail-Beigi, F., 19
 Ittensohn, O.L., 332
 Iwahara, S., 102
 Iwami, Y., 360
 Iyengar, J.R., 20
 Jack, C.W., 238
 Jackson, W.P.U., 77
 Jacob, M., 337
 Jacobs, A., 14, 105
 Jacobs, B., N287
 Jacobsen, J.G., 347
 Jacobson, L.O., 69
 Jager, F.C., 167
 Jägerstad, M., B319
 Janowski, S., 189
 Jansen, H.M., 360, 361
 Japlon, J.M., 359
 Jarrett, E.E.E., 268
 Jelliffe, D.B., 43, 236
 Jelliffe, E.F.P., 43
 Jenkins, G.N., 359
 Jensen, S.B., 359
 Jepson, J.B., 296
 Johanssen, I., 360

- Johnson, B.C., 28
 Johnson, D.E., 20
 Johnson, F.R., 329
 Johnson, J.L., 186
 Johnson, W.J., N288
 Johnston, J.M., 87
 Jolly, S.S. 77
 Jones, K.M., 117
 Jones, L.A.P., 260
 Jones, S.B., 47
 Jonxis, J.H.P., 238
 Jonxix, J.H.P., 208
 Jordan, H.V., 360
 Jorfeldt, L., 297
 Jorgensen, N.A., 5
 Josephson, B., N285
 Jost, J.P., 58
 Joubert, C.P., 297
 Joynson, D.H.M., 105
 Jukes, T.H., 295
 Jul, M., B351
 Jung, W.F., 238
 Jütting, G., 101
- Kahn, H.A., 197
 Kakehashi, S., 359
 Kalafer, M.E., 135
 Kalan, E.B., 47
 Kalas, J.P., N287
 Kalbfleisch, J.M., 197
 Kamen, B., 11
 Kaminetzky, H.A., 299
 Kammer, R.B., 36
 Kanfer, J.N., 189
 Kapikian, Z.A., 332
 Kaplan, E., 69
 Kapsimalis, B., 359
 Kapur, J., 43
 Karimian, N., 19
 Kark, J.A., 219
 Karlberg, P., 109
 Karlsson, I., 218
 Karmarkar, J., 238
 Karmarkar, M.G., 43
 Kaser, M.M., 296
 Kass, E.H., N286
 Katsan, I.M., 140
 Kattab, A., 134
 Katunuma, N., 22
 Kaufman, R.M., 69
 Kayden, H.J., 166, 208
 Kazarinoff, M.N., 102
 Kaziyo, Y., 87, 101
 Keating, J., 167
 Keen, H., 197
 Kelleher, J., 166
 Keller, H.J., 87
 Kellerman, G.M., 87
 Kellock, T.D., 326
 Kelly, W.A., 308
 Kelperi, H., 172
 Kemp, M., 166
- Kempson, R.L., 42
 Kennaway, E.L., 137
 Kennaway, N.M., 137
 Kenrick, K.G., 268
 Kerp, L., 197
 Kerr, G.R., 94
 Kerrigan, J.H., 360
 Kesterson, J.W., 308
 Keyes, P.H., 359, 360
 Khurana, C.M., N287
 Kim, Y., 40
 Kimball, D.A., 309
 Kimball, O.P., 272
 Kimberg, D.V., 85
 King, E.J., 260
 King, R.A., 167
 Kirchgessner, M., 68
 Kirwan, W.O., 72
 Kishimoto, Y., 219
 Kishineff, S., 166
 Kissenger, H.A., 234
 Kitchen, J.L., 145
 Kitchens, R.L., 87
 Kjellstrand, C.M., N288
 Klaeveman, H.L., 206
 Klein, J.P., 360
 Klein, R.I., 169
 Kleinberg, I., 359
 Kleinman, H.K., 245
 Klevay, L.M., 309
 Klipstein, F.A., 36, 37
 Knappe, J., 101
 Knight, B.C.J.G., 296
 Knittle, J., 216
 Knoettner, P., 359
 Knutsen, C., 267
 Koch, B.A., 68
 Kodicek, E., 5, 210, 296
 Koepke, K.R., 80
 König, K.G., 359
 Koniusky, F.R., 68
 Kopple, J.D., 202
 Kopsa, H., N288
 Korman, M.G., 204, N285
 Korth-Schutz, S., 265
 Koutras, D.A., 172
 Kouvalainen, K., 68
 Kovalsky, V.V., 69
 Krasse, B., 359
 Krebs, H.H., 87
 Krichevsky, M.I., 360
 Krimsky, I., 25
 Krishnamachari, K.A.V.R., 77
 Krishnaswamy, K., 22, 77
 Krook, L., 5
 Krugman, S., 332
 Krumdieck, C.L., 117
 Kruse, R., N223
 Kuhlenschmidt, M.S., 190
 Kulapongs, P., 105
 Kumar, S., 271
 Kuramitsu, H.K., 359
- Kvorning, S.A., 261
 Kyraikides, E.C., 204
- LaBrec, E.H., N287
 Labuza, T.P., B351
 Ladizesky, M., 5
 Lake, G.A., 166
 Lam, R.W., 94
 Lamanna, C., 122
 Lamb, M.W., B31
 Lamberg, B.A., 69
 Lambert, E.H., N288
 Lamego, C., 110
 Lane, M.D., 101
 Langer, A., 299
 Langley, F.A., 146
 Langston, J.W., 69
 Large, P.J., 117
 Larrson, C., 190
 Larsne, P.R., 302
 LaRusso, N.F., 204
 Laster, L., 186
 Latham, M.C., B256
 Lattimer, C., 332
 Launay, M., 238
 Lavau, M., 188
 Lawlor, G.J., Jr., 111, 337
 Lawson, D.E.M., 5
 Layrisse, M., 214, 240
 Leach, S.A., 359, 360
 Lease, J.G., 19
 Lee, G.R., 14
 Lee, H.J., 68
 Lee, H.M., 102
 Leevy, C.M., 299
 Lefèvre, A.B., 110
 Leitis, J., 302
 Lemaire, A., 261
 Leonard, P.J., 302
 Leonardopoulos, J., 172
 Leone, E., 87
 Leone, L.A., 270
 Lepkovsky, S., 295
 Leppla, W., 302
 Leslie, G.I., 117
 Lesser, G.T., 8
 Leu, J.A., 166
 Levenberg, B., 101
 Levi, A.J., 217
 Levin, S., 165
 Levine, R.A., 134
 Levinson, F.J., B95
 Levitt, M.D., 326
 Levy, D., 270
 Levy, R.I., 175
 Lewis, M., 56
 Lewy, J.E., 265
 Lezius, A., 101
 Li, C.H., 265
 Li, H.C., 101, 102
 Li, L.F., 101, 102
 Li, T.K., 217

- Liberfarb, R., 360
 Libonati, J.P., N287
 Lichtenstein, H., 109
 Lieber, C.S., 80
 Lifshitz, F., N223
 Lifton, S.D., 37
 Lijinsky, W., 20
 Likuski, H.J., 25
 Lim, S., 360
 Lindblad, B.S., 43
 Lindenbaum, J., 36
 Lipschitz, D.A., 14
 Littleton, N.W., 359
 Litwack, G., 216
 Liu, Y.K., 37
 Livingston, S., N223
 Llewellyn, P., 14
 Llory, H., 360
 Lloyd, O.C., 62
 Löe, H., 359
 Loening, W.E.K., 111, 336
 Loeper, J., 261
 Logan, R.W., 268
 Lojkin, M.E., 296
 Lombeck, I., 329
 London, W.T., 172
 Long, H., 146
 Longmire, R., 111
 Lonning, J.E., 234
 Lorch, E., 101
 Losowsky, M.S., 166
 Lott, I.T., 189
 Lovdal, A., 359
 Lowell, R.A., 22
 Lowenstein, F.W., 236, LE350
 Lowenstein, J.M., 55
 Lucy, J.A., 166, 208
 Ludwig, J., 69
 Ludwig, T.G., 140
 Lufkin, E.G., 58, 329
 Luft, R., 265
 Lumb, M., 120
 Lumeng, L., 217
 Luyken, R., LE191
 Lyman, R.L., 166
 Lynd, F.T., 5
 Lynen, F., 87, 101
 Lyon, J.B., Jr., 216

 McAleer, W.J., 332
 McCall, N.E., 102
 MacCardle, R.C., 261
 McCarrison, R., 172
 McCay, C.M., 241
 McCay, P.B., 166, 208
 McClellan, G.S., 296
 McClure, J.P., 268
 McCollum, E.V., 48
 McCombs, H.L., 110
 McCormick, D.B., SA97, 101, 102
 McConnell, A.A., 72
 McCoy, K., 205

 McDermott, W., N317
 MacDonald, I., 45
 MacDonald, J.B., 359
 MacDonald, T.T., 268
 McDougal, W.A., 360
 McGandy, R.B., 45
 McGanity, W.J., 296
 McGarry, J.D., 349
 McGill, D.G., 40
 McHugh, W.D., 359, 360, 361
 McLaren, D.S., 236
 McLean, F., 8
 McLean, P., 182
 McMasters, V., 165
 McMillan, R., 111
 McMin, R.M.H., 329
 McNutt, K.W., SA289, 359
 McQueen, R.W., 72, 326
 MacWilliam, K.M., 302
 Maccioni, A., 302
 Macdougall, L.G., 166
 Machado, D.V.M., 110
 Maclaren, N.K., N223
 Macy, I.G., 296
 Madden, R.J., 295, 296
 Madeira, M.C., 180
 Mafoyan, A., 111, 336
 Magee, P.N., 20
 Mahler, R., 56
 Majaj, A.S., 134, 165
 Mäkinen, K.K., 359, 360
 Malamos, B., 172
 Malan, A.F., 271
 Man, N.K., N288
 Mangelsdorg, P.C., 128
 Mangla, J.C., 40
 Manly, R.S., 360
 Mann, D.L., 146
 Mann, J.I., 45
 Marble, A., 198
 March, B.E., 166, 270
 Marcondes, E., 110
 Marcus, F.I., 270
 Margie, J.C., B256
 Marie, P., 210
 Marine, D., 272, 338
 Marks, J., 145
 Markstein, R., 166
 Marsh, A., 146
 Marshall, E.M., 197
 Marston, H.R., 68
 Martin, M.P., 296
 Martin, P.E., 126
 Martin, P.G., 68
 Martin, W.G., 122, 346, 347
 Martinez-Torres, C., 214, 240
 Maruyama, H., 197
 Masawe, A.E.J., 105
 Mascoli, C.C., 332
 Masironi, R., 135
 Mason, D.Y., 166
 Mason, J.B., 296

 Masoro, E.J., 281
 Massey, B., 36
 Mathan, V.I., 334
 Mathur, M., 334
 Matoush, L.O., 69
 Matthews, D.M., B319
 Mattingly, S.J., 361
 Mautalen, C.A., 5
 Maxiz, V., 135
 Maxwell, J.D., N223
 Mayer, J., 25
 Maynard, L.A., 241
 Mayne, V., 189
 Mayson, S.M., 145
 Mazumder, R., 87
 Medalie, J.H., 197
 Melhorn, D.K., 166, 208
 Mellette, S.J., 270
 Meloni, S., 135
 Melville, M.R.B., 359
 Melville, T.H., 360
 Menaker, L., 180
 Mendeloff, A.I., SA321
 Menkes, J.H., 94, 189
 Mentendiek, M.A., 182
 Menzel, D.B., 166
 Mergenhagen, S.E., 360
 Merrill, R., 210
 Mertz, E.T., B191
 Mertz, W., SA129, 134, 135
 Mess, B., 91
 Metz, J., 219
 Metz, R., 198
 Meulemans, O., 191
 Meyer, K.F., N317
 Meyers, J.A., 122
 Mezey, E., 37
 Michaelsson, G., 329
 Middleton, F.G., 36
 Miki, E., 197
 Miles, L.E.M., 14
 Miles, W.F., 20
 Miller, C.H., 361
 Miller, D.S., 236
 Miller, F., 14
 Miller, F.M., 14
 Miller, J., 296
 Miller, O.N., 55, 296
 Miller, R.F., 68
 Miller, W.J., 332
 Mills, C.F., 5, 69
 Millward, D.J., 76
 Milne, D.B., 260
 Milner, R.D.G., 91, 302
 Milstead, R.R., 8
 Mistry, S.P., 101
 Mitchell, W.D., 72, 326
 Mitman, F.W., 135
 Mitra, R.C., N287
 Mitschke, H., N288
 Mizuno, T., 22, 23, 58
 Molenaar, I., 166, 208

- Monckeberg, F., 91
 Monsen, E., 214
 Monson, E.R., 240
 Moore, C.V., 240
 Moore, F., 56
 Moraitopoulos, C., 172
 Morganroth, J., 175
 Morii, T., 68
 Morris, C.A., N287
 Morris, R.S., 56
 Morsey, M.R.A., 43
 Morton, J.F., 5
 Moser, H.W., 219
 Moss, J., 101, 190
 Moyer, E.Z., 296
 Moynahan, E.J., 329
 Mudd, S.H., 186
 Mueller, P.K., 166
 Mühlemann, H.R., 359
 Muindi, J.M., 105
 Mukasa, H., 359, 360
 Muller, M.C., 325
 Muller, S.A., 260
 Munves, E.D., 299
 Muratan, S., 68
 Murray, E., 182
 Murthy, G.K., 68
 Murthy, L., 309

 Naeye, S.L., 111
 Nagata, N., 261
 Nagy, M.E., B31
 Nair, M.G., 117
 Nair, P.P., 166, 208
 Naismith, D.J., 45
 Nalbandian, J., 361
 Narasinga Rao, B.S., 296
 Narisawa, K., 58
 Nason, A.P., 68
 Nathaniel, R.S., 245
 Nathanielsz, P.N., 302
 Navia, J.M., 180
 Naylor, M.N., 359
 Neldner, K.H., 329
 Nelsestuen, G.L., 28
 Nelson, R.A., 69, B256
 Nettesheim, P., 20
 Neufeld, H.N., 197
 Neumann, C.G., 111, 337
 New, M.I., 265
 Newberne, P.M., 20
 Newbill, J.A., 296
 Newbrun, E., 359, 360
 Newton, C., 337
 Newton, G., 22
 Nichols, B.L., 76
 Nicol, B.M., 296
 Nikaido, H., 361
 Nikkila, E.A., 200
 Niles, G.M., 296
 Nitowsky, H.M., 165
 Noble, A., 7

 Nockles, C.F., 166
 Noguchi, T., 166
 Nordén, A., B319
 Nordoy, A., B32
 Northfield, T.C., 171
 Numa, S., 101
 Nygaard, M., 359

 Oberleas, D., 267
 O'Brien, A., 360
 Ochoa, S., 87, 101
 O'Connell, D.E., 236
 Odake, S., 295
 O'Dell, B.L., 19
 Oelshlegel, F.J., Jr., 267
 Oettinger, C.W., 210
 Oettle, G.J., 219
 Ogle, P.J., 296
 Oh, W., 94, 111, 169
 O'Halloran, M.T., 91
 Ohlson, M.A., 45, 198
 Okos, A., 111
 Olatunbosun, D., 69
 Olefsky, J.M., 200
 Olson, R.E., 105, 166
 Olsson, I., 359
 Olsson, K.S., 14
 Omaye, S.T., 166
 Omdahl, J.F., 5
 Omori, K., 94
 Oppenheimer, E.H., 165
 Oppen, A., 299
 Ortega, J., 267
 Osborne-White, W.S., 120
 Osborne-White, W.W., 117
 O'Shaughnessy, M.C., 14
 Oski, F.A., 145, 165
 Overath, P., 87
 Owen, C.A., Jr., 53
 Owen, O.E., 302
 Oxnard, C.E., 219

 Painter, N.S., 72, 326
 Palframan, J.F., 20
 Panalaks, T., 20
 Panasiuk, O., 47
 Papageorgiou, A., 169
 Papapetrou, P.D., 172
 Parent, M.A., 11, 336
 Parikh, H.C., 284
 Parizkova, J., 8
 Park, J., 270
 Parker, J.C., 135
 Parker, R.B., 361
 Parman, G.K., 167
 Parrott, D.M.V., 268
 Parsa, A., 19
 Parsa, M.H., B32
 Passmore, R., 296
 Pattinian, H., 25
 Patwardhan, V.N., 134, 135
 Pauli, L.L., N223

 Paunio, I.K., 360
 Payne, J.W., B319
 Payne, P.R., 236
 Peake, I.R., 166
 Pearce, M.L., 74
 Pearson, J.R., 326
 Pechet, M., 261
 Pechet, M.P., 210
 Peeler, J.T., 68
 Pekarek, R.S., 134
 Pelc, B., 210
 Pelikan-Filipova, M., 238
 Pelroy, G.A., 122
 Pena, A.S., 36
 Pereira, S.M., 165
 Peres, G., 260
 Pernow, B., 297
 Perry, J., 120
 Peskin, M.R., 22
 Petering, H.G., 309
 Peterlik, M., 5
 Peters, R.A., 211
 Peters, S.P., 190
 Petersen, K., 197
 Petersen, R.J., N288
 Peterson, M.L., 238
 Peterson, P.A., 245
 Pezzimenti, J.F., 36
 Pfeifer, P.M., 166, 208
 Phillips, G.B., 166
 Phillips, I., 337
 Philosophy, L., 359
 Pike, R.L., B320
 Pildes, R.S., 271
 Pisano, J.J., 277
 Plank, P., 5
 Plasschaert, A.J.M., 359
 Platt, B.S., 43
 Plzak, L.F., 69
 Pocker, A., 217
 Polakis, S.E., 101
 Polansky, M.M., 134
 Pollack, S., 69
 Pollock, M., N288
 Ponniah, J., 334
 Pope, A.L., 166
 Posner, A.S., 260
 Potter, D.E., 265
 Potter, F.L., 107
 Poukka, R.K.H., 165
 Poukka Evarts, R., 167
 Poysky, F.T., 122
 Pozefsky, T., 302
 Prasad, A.S., 267
 Prasad, S., 77
 Pressman, A., 172
 Price, J.M., 296
 Price, M.G., 134
 Price, N.O., 68
 Price, W.R., 22
 Prohaska, J.R., 308
 Prout, T.E., 302

- Prout, W., 112
 Provost, P.J., 332
 Pugh, E., 216
 Purcell, R.H., 332
- Quarentei, G., 110
 Quaresimo, A., 167
 Quayle, J.R., 117
- Raben, M.S., 265
 Raghuramulu, N., 296
 Rahimtoola, R., 43
 Rahm, J.J., 331
 Raibaud, P., 325
 Rajagopalan, K.V., 186
 Rajalakshmi, R., B256
 Ramachandran, K., 334
 Ramakrishnan, C.V., 43
 Ramalingaswamy, V., 334
 Ramanathan, M.K., 43
 Ramsey, H.A., 206
 Rana, I.A., 45
 Ransome-Kuti, O., 134
 Rao, M.N., 296
 Rasmussen, H., LE31, 210, 261
 Rathi, M., 271
 Raud, W.M., 182
 Raulin, R., 238
 Rawlings, W., 190
 Rayfield, E.J., 134
 Raz, A., 245
 Reaven, G.M., 200
 Reba, R.C., 69, 134
 Reddy, B.S., 137, 326
 Reddy, K., 166
 Reddy, M.V., 22
 Redinger, R.N., 74
 Register, U.D., 296
 Reh fuss, M.E., 325
 Reinhold, J.G., 19
 Reissner, S.H., 169
 Reiss, U., 166
 Rekant, S.I., 135
 Renaud, S., B32
 Reynell, P.C., 268
 Reynolds, J.J., 217
 Reynolds, M.S., 296
 Rhea, U., 68
 Rhoades, J.W., 20
 Richard, M., 238
 Richardson, D.T., 360
 Richens, A., N223
 Richert, D.A., 186
 Rickenberg, H.V., 58
 Rickes, E.L., 68
 Riggio, R.R., 265
 Rigler, L.G., N317
 Rigopoulos, G.A., 172
 Ringelmann, E., 101
 Riss, E., 197
 Ritchie, A.E., N287
 Ritchie, J.H., 165
- Ritz, H.L., 359, 361
 Ritzl, F., 329
 Robertson-Smith, D., 87
 Robles, E.A., 37
 Rodriguez, J., 76
 Roehm, J.N., 166
 Roepstorff, P., 28
 Rogers, A.E., 20
 Rogers, H.J., 105
 Roginski, E.E., 134, 135
 Rollier, M.A., 135
 Roncevic, N.P., 145
 Roon, R.J., 101
 Root, W.S., 68
 Rosan, B., 360
 Roscoe, J.D., 145
 Rose, W.C., 202
 Rosen, S., 359
 Rosenfield, S., 216
 Rosensweig, N., 37
 Rosensweig, N.S., 58
 Rosenthal, H.L., 296, 303
 Rosner, B., N286
 Ross, E., 5
 Roth, J.A., 102
 Rothenberg, S.P., 11, 184
 Rothwell, W.S., 296
 Rotruck, J.T., 166
 Rowe, D.J.F., N223
 Rowe, V.D., 277
 Rowland, L.O., Jr., 5
 Rubenstein, H.M., 166
 Rubin, A.L., 265
 Rubin, C., 36
 Ruck, B.J., N287
 Rucker, R.B., 308
 Ruffin, J.M., 296
 Ruis, H., 101, 102
 Rulli, M.A., 180
 Rummel, R., 68
 Rundles, R.W., 214
 Russell, M., 238
 Russell, R.I., 326
- Sack, R.B., N287
 Sacks, F.M., N286
 Saenger, P., 265
 Sainsbury, I.S., B31
 Sakai, K., 22, 23
 Sakami, W., 117, 219
 Saliternik, R., 197
 Salmon, W.D., Jr., 265
 Sands, J., 7
 Sandstead, H.H., 80
 Saner, G., 134, 135
 Sansom, B.F., 5
 Sapers, G.M., 47
 Sarett, H.P., 296
 Sarma, R., 77
 Sarri, J.C., 217
 Sasakawa, T., 87
 Saslaw, M.S., 359
- Sass, N.L., 346
 Sato, G.H., 219
 Sauberlich, H.E., B31, 297
 Sausse, A., N288
 Sawyer, R., 20
 Saxton, C.A., 359, 360, 361
 Scallen, T.J., 205
 Schachtele, C.F., 359, 360
 Schade, A.L., 105
 Schade, D.S., 200
 Sharp, C.R., 210, N223
 Schatzki, P., 205
 Schenk, E.A., 37
 Scherp, H.W., 360
 Schertz, L.P., 234
 Schiff, D., 169
 Schimke, R.T., 182
 Schlegel, H.G., 101
 Schlenk, F., 296
 Schmid, F., N223
 Schmidt, P., N288
 Schmidt, S.Y., 346
 Schmitthenner, U., 197
 Schneider, D.L., 346
 Schneider, H.A., 198
 Schneidman, K., 216
 Schnippering, H.G., 329
 Schonland, M., 111, 336
 Schoomaker, E.B., 267
 Schreiber, R.A., 23
 Schroeder, H.A., 68, 135, 140
 Schroeder, H.E., 359
 Schultz, H.W., 47
 Schuster, M.W., 205
 Schwartz, D., 189
 Schwartz, E., 265
 Schwarz, K., 134, 135, 260, 261
 Schwieter, U., 166, 167
 Scott, H.W., Jr., 80
 Scott, M.L., 166
 Scott, R.B., 36
 Scow, R.O., 206, 343
 Scrimshaw, N.S., 7, 182, 336
 Scliver, C.R., 169
 Scrutton, M.C., 58, 101
 Scullard, G.S., 76
 Searcy, M.T., 166
 Sebrell, W.H., 165, 166, 331
 Seddick, Y., 43
 Sederholm, T., 68
 Seeman, A., 198
 Seier, L., 166, 270
 Seiler, D., 281
 Selhub, J., 117
 Selvey, N., B351
 Sen, N.P., 20
 Sered, B.R., 145
 Serjeant, G.R., 267
 Setian, N., 110
 Shaftel, R., 238
 Shambaugh, G.E., III, 91
 Shami, S.W., 135

- Shapiro, S., 216
 Shea, N., 36
 Sheahan, D.G., N287
 Shearer, T.R., 140
 Sherman, A.R., 56
 Sherwood, W., 36
 Shideman, J.R., N288
 Shields, G.S., 309
 Shils, M.E., 271
 Shimamura, T., 295
 Shin, Y.S., 117
 Shiner, M., 268
 Shiota, T., 117
 Shipley, P.G., 48
 Shively, J.N., 5
 Shmerling, D.H., 268
 Shockman, G.D., 361
 Shoemaker, W.C., 284
 Shreiber, C., 184
 Shute, E.V., 166
 Sibinovic, K.H., 172
 Siddons, R.C., 219
 Sigel, H., 101
 Siimes, M.A., 14
 Silber, R., 208
 Silberfarb, P.M., 80
 Sim, J., 166, 270
 Simmonds, N., 48
 Simmonds, W.F., N285
 Simmonds, W.J., 204
 Simmons, J.J., 198
 Simmons, J.M., 265
 Simon, U., 5
 Simpson, C.F., 5
 Sinclair, A.J., 281
 Singh, D., 236
 Singhal, R.L., 182
 Sink, J.S., B32
 Sinnhuber, R.O., 47
 Sipple, H.L., 359
 Sites, M.D., 146
 Sjögren, I., 109
 Skala, J.H., B31, 297
 Sklair, I.R., 360
 Skoryna, S.C., 68, 69
 Slade, H.D., 359, 360
 Sleisinger, M.H., 268
 Slover, H.T., 167
 Small, D.M., 74, 204
 Small, M., 87
 Smith, A.N., 72, 326
 Smith, C.L., 166
 Smith, D.C., 140, 167
 Smith, D.T., 296
 Smith, E.L., 68
 Smith, G.W., 68
 Smith, L.H., Jr., 347
 Smith, R., 76
 Smith, R.M., 117, 120
 Smith, S.E., 68
 Smith, S.G., 296
 Smith, S.R., 302
 Smith, V.E., B319
 Smith, W.T., 219
 Smoak, W.M., 36
 Smythe, P.M., 111, 336, 337
 Snyder, C., 20
 Snyder, M.J., N287
 Snyderman, R., 360
 Socransky, S.S., 359
 Sökücü, S., 135
 Solow, C., 80
 Sols, A., 101
 Sood, S.K., 334
 Souma, L.M., 135
 Southgate, D.A.T., 72, 326
 Spaeth, D.G., 346
 Spagnuolo, C., 281
 Sperling, G., 241
 Sperling, M.A., 169
 Spies, A., 5
 Spina-France, A., 110
 Spiro, H.M., 165
 Spitz, F.J., 281
 Spitzer, A., 107
 Spitzer, H.L., 205
 Sprague, E.W., B191
 Spriggs, A.I., 36
 Spritz, N., 197
 Srikantia, S.G., 22, 296
 Srinivasan, R., 166
 Srinivasan, P.R., 43
 Staat, R.H., 360
 Stadtman, E.R., 87
 Stakman, E.C., 128
 Stamm, N.B., 182
 Stanbury, J.B., 172, 296
 Stangherlin, T., 110
 Stanley, H.R., 360
 Stare, F.J., 45, 238, B351
 Stearns, G., 296
 Stebbing, G.E.T., 172
 Steigleder, G.K., 189
 Steinberg, D., 197
 Stenflow, J., 28
 Stenzel, K.H., 265
 Stephen, J.M.L., 182
 Stephens, M.C., 22
 Stern, L., 169
 Sterner, R.T., 22
 Stevens, A.R., Jr., 14
 Stevens, B.H., 189
 Stewart, C.N., 22, 23
 Stewart, D.A., N223
 Stewart, P.L., 68
 Stiehm, E.R., 111, 337
 Stierwalt, E., 146
 Stifel, F.B., 58, 329
 Stinson, D.A., 302
 Stipe, W.H., 208
 Stock, A.L., 45
 Stockell, A., 296
 Stocks, J., 166
 Stoffel, W., 238
 Stokstad, E.L.R., 117, 120, 219
 Stoll, E., 101
 Stone, D.B., 198
 Storvick, C.A., 140
 Stotz, E.H., 101
 Streeten, D.H.P., 134, 135
 Strong, F.M., 295, 296
 Stubbe, P., 302
 Stumpf, S.E., 234
 Sturdevant, R.A., 74
 Sturman, J.A., 216
 Subramanian, C., 281
 Suchkov, B.P., 140
 Sukhatme, P.V., 43, 236
 Sullivan, A.C., 55
 Sullivan, J.F., 69
 Sullivan, L.W., 37
 Summerskill, W.H.J., 171
 Sumners, J.E., 8
 Sun, A.Y., 281
 Sun, G.Y., 281
 Sunde, R.A., 166
 Sundler, R., 205
 Sung, J.H., 189
 Suskind, R., 91, 105
 Sussman, K., 198
 Sutter, V.L., 326
 Suttie, J.W., 28, 134, 167
 Suzuki, U., 295
 Svennberg, I., 109
 Svennerholm, L., 281
 Swai, G.B.R., 105
 Swaminathan, M.C., 236
 Swanson, A.B., 166
 Swanson, V.L., 36
 Swarnabai, C., 334
 Sweetnam, P., 56
 Sweetnam, P.M., 334
 Swell, L., 205
 Swenseid, M.E., 202, 337
 Swenson, R.S., 42
 Swift, K., 80
 Szabo, A.J., 238
 Szoz, K.F., 210
 Tabor, H., 58
 Tainter, M.L., 326
 Talley, F.B., 47
 Tamm, R., 167
 Tamura, T., 22, 23, 58, 117
 Tank, G., 140
 Tanner, J.M., 8
 Tanno, K., 58
 Tanzer, J.M., 360
 Tappel, A.L., 166, 208
 Tarka, S., 346, 347
 Tarka, S.M., 347
 Tashjian, A.H., 5
 Tatevossian, A., 359
 Tatsumi, S., 22, 23
 Taunton, O.D., 58
 Taussig, H.B., N317

- Taylor, A.N., 5
 Taylor, C.E., 336
 Taylor, D.W., 166
 Taylor, H.C., Jr., N318
 Taylor, H.W., 20
 Taylor, R.F., 68
 Teitelbaum, A., 197
 Tempel, T.R., 360
 Tengerdy, R.P., 166
 Terris, M., 296
 Tevetoglu, F., 68
 Tews, J.K., 22
 Theilade, E., 359
 Thenen, S.W., 120
 Thiele, V.F., 216
 Thierstein, S.T., 107
 Thodenius, K., 107
 Thomas, H.V., 166
 Thomassen, R.W., 36
 Thompson, J.F., 68
 Thompson, J.N., 140, 167
 Thompson, R.G., 91
 Thompson, R.H.S., 211
 Thomson, A.D., 299
 Thorburn, G.D., 94
 Thyresson, N., 329
 Tierney, D.F., 166
 Tilley, J.M., 5
 Tipton, I.H., 68
 Todhunter, E.N., SA289, 296
 Toepfer, E.W., 134
 Tokarnia, C.H., 5
 Toskes, P.P., 68
 Touster, O., 296
 Tove, S.B., 206
 Toverud, K.U., 296
 Treble, D.H., 204, 205
 Trier, J.S., 36, 37
 Triscaria, J., 55
 Troelstra, J.A., 208
 Trowell, H., 197
 Trowell, H.C., 72, 297
 Troy, J.L., 107
 Truelove, S.C., 36, 268
 Truex, R., 346, 347
 Truswell, A.S., 45
 Tsuji, S., 197
 Tuman, R.W., 134
 Turco, S.J., 190
 Turk, K.L., 68
 Turner, J.M., 217

 Ukstins, I., 219
 Underwood, E.J., SA65, 68, 260, 308
 Unger, R.H., 200
 Unglaub, W.G., 296, 303
 Uribe, A., 5
 Urone, P., 69
 Usher, R., 8
 Utiger, R.D., 91
 Utter, M.F., 58

 Vaes, G., 23
 Vagg, M.J., 5
 Vague, J., 197
 Vahlquist, B., B32, 109
 Valberg, L.S., 69
 Valente, M.I., 110
 Vallada, L.P., 110
 Van den Brande, J.L., 265
 van der Haven, J.S., 359
 van Houte, J., 359, 360, 361
 Van Itallie, T.B., 165
 Van Soest, P.J., 72, 325
 van Tonder, S.V., 219
 Van Wyk, J.J., 265
 Varga, F., 91
 Venkatachalam, P.S., 43
 Vickery, H.B., 295
 Victor, M., 219
 Vidal, A.J., 120
 Vietsch, R.L., 217
 Vijaya Raghavan, K., 236
 Villarejos, V.M., 332
 Visser, H.K.A., 208
 Viteri, F., 76
 Vithayasai, V., 105
 Vivian, V.M., 296
 Vlachcevic, Z.R., 205
 Vogelsang, A.B., 166
 von Euler, H., 296
 von Hartitzsch, B., N288
 Vos, G.H., 111, 336
 Vos, J., 166, 208
 Vought, R.L., 172

 Wada, H., 22
 Wada, M., 197
 Wada, Y., 58
 Waddell, J., 47
 Waerhaug, J., 359
 Wahren, J., 297, 302
 Wakil, S., 101
 Walker, A.R.P., 43, 326
 Walker, B.E., 166
 Walker, D.M., 105
 Walker, G.J., 360
 Walker, J.G., 326
 Walker, M., N318
 Walker, R., 214
 Walker, R.B., 240
 Walker-Smith, J.A., 268
 Walling, M.W., 85
 Walters, G.O., 14
 Wangenstein, O.H., N318
 Wannemacher, R.W., Jr., 134
 Warburg, O., 296
 Ward, E.E., 76
 Wardrop, C.A., 14
 Warner, D.T., 202
 Warren, S., N318
 Wase, A.W., 5
 Washburn, T.C., 69

 Waslien, C.I., 135
 Wasserman, R.H., SA1, 5
 Watanabe, S., 22
 Waterlow, J.C., 182
 Waters, W.E., 334
 Watson, C.J., N318
 Watson, J.A., 55
 Waxman, S., 36, 184
 Weakley, D.R., 189
 Wearn, J.T., N318
 Weber, G., 182
 Webster, H.L., 91
 Weetall, H., B256
 Weidel, H., 295
 Weidemann, M.J., 87
 Weight, L.A., 5
 Weinberg, T., 165
 Weinsier, R.L., 198
 Weinstein, M.B., 36
 Weiser, H., 167
 Weisinger, J.R., 42
 Weissbach, H., 117
 Weller, J.M., 186
 Wells, W.W., 308
 Welsby, E., 56
 Wene, J.D., 331
 Wertz, A.E., 296
 West, K.A., SA193, 197, 198
 Westerfeld, W.W., 186
 Wharton, B., 337
 Wheatley, V.R., 55
 Wheby, M.S., 36
 Whelan, E.M., B351
 White, A.M., 87, 219
 White, P.H., 349
 White, P.L., N30, B31, B160, B351
 Whitehead, R., 36
 Whitehead, R.G., 337
 Whitehouse, R.H., 8
 Whitfield, C.D., 117
 Whiting, G.C., B351
 Whyte, H.M., 8
 Widdowson, E.M., 238
 Wiedemann, E., 265
 Wieland, R.G., 80
 Wiese, H.F., 331
 Wiggins, H.S., 326
 Wilber, J.F., 91
 Wilkins, J.W., 186
 Wilkins, T.D., 137
 Willcock, E.G., 15
 Willers, E.H., 5
 Williams, C., 296
 Williams, H.L., 216
 Williams, M., 219
 Williams, M.A., 117
 Williams, P.R., 169
 Williams, R.V., 69
 Williams, W.J., 214
 Williams, W.P., Jr., 19
 Williams, W.W., 338
 Willis, M.R., 62

Willison, J.W., 270
 Wilmore, D.W., 271
 Wilson, A.B., 105
 Wilson, C.J., 265
 Wilson, R.F., 359
 Winawer, S.J., 37
 Windmueller, H.G., 166
 Winick, M., 7, B320
 Winter, R., 208
 Wintrobe, M.M., 214, 309, N318
 Wirtz, C.W., 36
 Wise, G.H., 206
 Wise, W.R., 329
 Wiss, O., 166, 167
 Witting, L.A., 166
 Wolanski, B.S., 332
 Wolf, G., 245
 Wolf, H., 302
 Wolf, R.O., 206
 Wolf, W.R., 135
 Wong, E., 166, 270

Wood, J.M., 359, 360
 Wood, T.R., 68
 Wood, W.B., Jr., N318
 Wood, W.I., 360
 Woodruff, C.W., 334
 Woolley, D.W., 295, 296
 Worker, N.A., 4
 Worwood, M., 14
 Wright, L.D., 101, 102
 Würsch, J., 166
 Wybregt, S.H., 169
 Wynder, E.L., 137
 Wyndner, E.L., 326
 Wyngaarden, J.B., 296
 Wyngarden, L., 58

Yalow, R.S., 265
 Yamada, L., 22
 Yamada, T., 360, 361
 Yamaguta, N., 68

Young, L.W., N223
 Young, V.H., 302
 Young, V.R., 182
 Younger, R.K., 80
 Yu, B.P., 281
 Yudkin, J., 45
 Yunis, E.J., 190

Zak, G., 8
 Zalusky, R., 219
 Zamcheck, N., 37
 Zazgornik, J., N288
 Zempi, J.W., 23
 Zetterström, R., 107
 Zimmerli, W., 166
 Zinder, O., 343
 Zinner, D.D., 359
 Zwergel, E.E., 101
 Zykovicz, T.H., 28

SUBJECT INDEX

The letters SA preceding the page number designate a special signed article and N refers to articles or letters in the Notes section.

ABSORPTION

- amino acids, by the crocodile, 281
- calcium
 - in ileum, 84
 - in renal disease, 209
 - in S. malacoxylon poisoning, SA1
- chromium salts, SA129
- cobalt, SA65
- copper, in steely-hair syndrome, N189
- fat, defect in, vitamin E deficiency in, SA161
- iron
 - in inflammatory diseases, 213
 - studies with isotopic iron, 238
- phosphorus, in S. malacoxylon poisoning, SA1
- silicon, SA257
- studies on, D-xylose approved as diagnostic agent, N320
- α -tocopherol polyethylene glycol succinate, 206
- xylose, in pernicious anemia, SA33
- zinc
 - from wholemeal breads, 18
 - in acrodermatitis enteropathica, 327

ACETYL CoA

- levels in liver, in vitamin B₁₂ deficiency, 85

ACHLORHYDRIA

- basis for intestinal malabsorption in pernicious anemia, SA33

ACRODERMATITIS ENTEROPATHICA

327

S-ADENOSYL METHIONINE

- in liver, in vitamin B₁₂ deficiency, 118

ADIPOSE

- tissue
 - composition, of infants, 236
 - fat synthesis in, effect of hydroxycitrate on, 53
 - glutathione peroxidase in, in selenium and vitamin E deficiency, SA161
 - lipoprotein lipase activity in, effect of prolactin on, 341

ADRENAL

- changes, in adult protein-calorie malnutrition, 239

ADVERTISING

- foods, proposed FTC regulations, N29, 246

AGING

- relation of silicon to, SA257
- relation to caloric intake (Nutrition Classic), 241

AGRICULTURE

- development, World Food Conference, SA225

ALBUMIN (see PROTEIN)

ALCOHOLISM

- relation to folate deficiency and intestinal absorption, SA33

ALKAPTONURIA

- nutrition classic, 81

ALPHA OMEGA ALPHA

- listing of interviews with leaders in American Medicine, N316

AMERICAN MEDICAL ASSOCIATION

- termination of Council on Foods and Nutrition, past contributions, N29, N64

AMINES

- role in cancer production by nitrosamines, 19

AMINO ACIDS

- alanine, response to feeding in small-for-gestational-age infants, 168
- association with chromium, in glucose tolerance factor, SA129
- blood levels
 - during total parenteral nutrition of low birth-weight infants, 270
 - in adults with protein-calorie malnutrition, 299
- cysteine, deficiency, taurine synthesis in, 343
- gamma-carboxyglutamic acid, in prothrombin, 25
- histidine, essential for adults, 200
- importance in metabolism (Nutrition Classic), 15
- leucine, effect on niacin requirement, review, SA289
- methionine, synthesis, in folate deficiency, 118
- phenylalanine, excretion of metabolites in experimental phenylketonuria, 276
- selenomethionine, availability of selenium in, 138
- taurine, role in retina and heart, 343
- tryptophan, precursor of niacin, review, SA289
- tryptophane, nutritional significance (Nutrition Classic), 15
- utilization by crocodiles, 281

AMINOPYRINE

- production of cancer from, 19

ANEMIA

- gestational, effect of iron supplementation on, 332
- histidine deficiency, 200
- inflammation, role of reticuloendothelial cells in, 213
- iron deficiency
 - hyperlipemia in, 55
 - lymphocyte function in, 103
- pernicious
 - bat as an experimental model, 217
 - xylose absorption in, SA33
- postnatal, iron deficiency as cause of, 144
- serum ferritin levels in, 11
- sickle cell, zinc deficiency in, 266
- vitamin E deficiency, 206
 - in premature infants, SA161

ANTERIOR PITUITARY

- control of lipoprotein lipase activity by, 341

ANTIBIOTICS (see DRUGS)

ANTIBODIES

- circulating, in celiac disease, 267
- tests, for diagnosis of viral hepatitis A, 331
- to insulin, effect on ketogenesis, 347
- to xanthine oxidase, 59

ANTIMETABOLITES (see DRUGS)

APPETITE

- effect of hydroxycitrate on, 53

- ARGENTINA**
enteque seco in, cattle disease from calcinogenic plants, SA1
- ASCORBIC ACID**
blood levels, in maternal and newborn, 298
oxidation, from supplemental iron in foods, 46
- ATHEROSCLEROSIS**
in diabetes, SA193
in type III hyperlipoproteinemia, 173
relation to silicon, SA257
- ATP**
levels in liver, in vitamin B₁₂ deficiency, 118
- AVAILABILITY**
chromium, in foods, SA129
energy, from dietary fiber, SA321
iron
relation to infection, 103
supplemental in foods, 46
zinc, in leavened and unleavened bread, 18
- BACTERIA**
Clostridium botulinum, bacteriophage for neurotoxin production, 120
colon, effect of dietary fiber on, SA321
E. coli, cause of diarrhea in children, N286
gram-negative infections, in malnourished children, 334
in dental plaque, relation to caries, SA353
in drinking water, production of antithyroid agents by, 171
intestinal flora, effect on sterol and bile acid excretion, 136
length of folic acid glutamyl side chains in, 115
overgrowth in intestine, basis for malabsorption in pernicious anemia, SA33
- BACTERIOPHAGE**
action on Clostridium botulinum to produce neurotoxin, 120
- BATS**
vitamin B₁₂ deficiency in, neurological damage in, 217
- BERI-BERI**
etiology (Nutrition Classic), 176
- BILE**
composition, relation to diet, 72
copper in, after feeding excessive copper, 51
secretion
dependence on caloric content of meal, 169
regulation of, 202
- BILE ACIDS**
adsorption to dietary fiber in the colon, SA321
excretion
relation to diet, 72, 136
test for liver function, N285
- BILIRUBIN**
blood levels, after jejuno-ileal bypass, 38
- BIOLOGIC ACTIVITY**
bound forms of niacin, review, SA289
chromium compounds, SA129
iron added to foods, 238
vitamin E analogues, SA161
- BIOTIN**
blood levels, in maternal and newborn, 298
deficiency, central nervous system abnormalities in, 21
review of role and biosynthesis, SA97
- BISULFITE**
toxicity, in molybdenum deficiency, 185
- BLOOD** (see under individual constituents)
- BODY COMPOSITION**
infants, by skinfold measurement, 7
- BODY WEIGHT** (see WEIGHT, BODY)
- BONE**
alkaline phosphatase in, during anticonvulsant drug therapy, 209, N221
aminolevulinic acid synthetase in, in vitamin E deficiency, SA161
copper in, after feeding excessive copper, 51
deposition, vitamin for (Nutrition Classic), 48
development, role of silicon on, SA257
diseases, use of vitamin D metabolites in, 209, N221
mobilization, in S. malacoxylon poisoning, SA1
osteosclerosis, in fluorosis, 76
- BOOK NOTICES**
A Study of Food Consumption by the Duplicate Portion Technique in a Sample of the Dalby Population. B. Borgström, A. Nordén and M. Jägerstad, Editors. The Almqvist and Wiksell Periodical Company, 319
Applied Nutrition. R. Rajalakshmi. Mohan Pramlani, 256
Aromatic Amino Acids in the Brain, Ciba Foundation Symposium 22. American Elsevier Publishing Company, Inc., 32
Body Dimensions and Proportions, White and Negro Children 6-11 Years, United States. Health Resources Administration, DHEW, 256
Chemicals and Health, Science and Technology Policy Office, National Science Foundation, 351
Childhood Obesity. M. Winick, Editor. John Wiley and Sons, Inc., 320
Chromium. National Academy of Science, 32
Cobalamin: Biochemistry and Pathophysiology. B.M. Babor, Editor. John Wiley and Sons, Inc., 320
Dietary Fats and Thrombosis, Proceedings of the INSERM Symposium. S. Renaud and A. Nordoy, Editors, S. Karger, 32
Early Malnutrition and Mental Development, Symposia of the Swedish Nutrition Foundation XII. J. Cravioto, L. Hambræus and B. Vahlquist, Editors. Almqvist and Wiksell, 32
Efficient Resource Use for Tropical Nutrition: Nigeria. V.E. Smith. Michigan State University Press, 319
Essentials of Food and Nutrition. Vol. 1-Fundamental Aspects. Vol. 2—Applied Aspects. M. Swaminathan. M.S. Venkatesan, 95
Food and Nutrition (1945-1972); Annotated Bibliography; Author and Subject Index. Unipub, 32
Geochemistry and the Environment: Volume 1, The Relation of Selected Trace Elements to Health and Disease. National Academy of Science, 32
High-Quality Protein Maize. L.F. Bauman, E.T. Mertz, A. Carballo and E.W. Sprague. Dowden, Hutchinson and Ross, Inc., 191
Immobilized Enzymes, Antigens, Antibodies and Peptides. H. Weetal, Editor. Marcel Dekker, Inc., 256
Improvement of Protein Nutrition. Food and Nutrition Board, National Research Council, National Academy of Sciences, 160

- Infant Nutrition. S.J. Fomon. W.B. Saunders Company, 32
- Laboratory Tests for the Assessment of Nutritional Status. H.E. Sauberlich, R.P. Dowdy and J.H. Skala. CRC Press, Inc., 31
- Lactic Acid Bacteria in Beverages and Food. J.G. Carr, C.V. Cutting and G.C. Whiting, Editors. Academic Press, 351
- Manganese. National Academy of Sciences, 32
- Morinda: An Economic Analysis of Malnutrition Among Young Children in Rural India. F.J. Levinson. Cornell/MIT International Nutrition Policy Series, 95
- National Institute of Nutrition, Annual Report, January 1, 1973 to December 31, 1973. Indian Council of Medical Research, 31
- Nickel. National Academy of Sciences, National Research Council, 351
- 1972 Evaluations of Some Pesticide Residues in Foods: The Monographs. Food and Agriculture Organization, 351
- Nutrients in Processed Foods. Proteins. P.L. White and D.C. Fletcher, Editors. Publishing Sciences Group, Inc., 160
- Nutrients in Processed Foods. Vitamins and Minerals. H.E. Bauman. Publishing Sciences Group, Inc., 31
- Nutrition: An Integrated Approach. R.L. Pike and M.L. Brown. John Wiley and Sons, Inc., 320
- Nutrition and Anti-Infectious Defense. I. Gontzea. S. Karger, 32
- Nutritional Assessment in Health Programs. G. Christakis, Editor. American Public Health Association, 32
- Orientations in Geochemistry. National Academy of Sciences, 32
- Panic in the Pantry: Food Facts, Fads and Fallacies. E.M. Whelan and F.J. Stare. Athenium, 351
- Peptide Transport in Protein Nutrition. D.M. Matthews and J.W. Payne, Editors. American Elsevier, 319
- Prevention of Microbial and Parasitic Hazards Associated with Processed Foods: A Guide for the Food Processor. Food and Nutrition Board, National Academy of Sciences, National Research Council, 319
- Proceedings of the Western Hemisphere Nutrition Congress IV. P.L. White and N. Selvey, Editors. Publishing Sciences Group, Inc., 351
- Protein and Nutrition Policy in Low-Income Countries. F. Aylward and M. Jul. Charles Knight and Company, Ltd., 351
- Protein Nutritional Quality of Foods and Feeds. Part 1. M. Friedman, Editor. Marcel Dekker, Inc., 319
- Safe Central Venous Nutrition: Guidelines for Prevention and Management of Complications. M.H. Parsa, J.M. Ferrer and D.V. Habib. Charles C. Thomas, 32
- Science and Technology in Presidential Policy-making. National Academy of Science, 32
- Skeletal Maturity of Children 6-11 Years, United States. Superintendent of Documents, U.S. Government Printing Office, 95
- Subunit Enzymes: Biochemistry and Functions. K.E. Ebner, Editor. Marcel Dekker, Inc., 320
- The Control of Metabolism. S. Sink, Editor. Pennsylvania State University Press, 32
- The Effects of Soils and Fertilizers on Human and Animal Nutrition. W.H. Allaway. Superintendent of Documents, U.S. Government Printing Office, 256
- The Liver: Normal and Abnormal Functions. F.F. Becker, Editor. Marcel Dekker, Inc., 191
- The Mayo Clinic Renal Diet Cookbook. J.C. Margie, C.F. Anderson, R.A. Nelson and J.C. Hunt. Western Publishing Company, Inc., 256
- The Meaning of Human Nutrition. M.L. Lamb and M.L. Harden. Pergamon Press, Inc., 31
- The Milk-Free and Milk-Free, Egg-Free Cookbook. I.S. Sainbury. Charles C. Thomas, 31
- The Nutrition Crisis: A Reader. T.P. Labuza. West Publishing Company, 351
- The Promotion of Bottle Feeding by Multinational Corporations: How Advertising and the Health Professions Have Contributed. T. Greiner and M.C. Latham, Editors. Cornell University, 256
- Total Parenteral Nutrition. P.L. White and M.E. Nagy, Editors. Publishing Sciences Group, Inc., 31
- Total Parenteral Nutrition: Premises and Promises. H. Ghadimi, Editor. John Wiley and Sons, 191
- Toxicological Evaluation of Certain Food Additives with a Review of General Principles and of Specifications. World Health Organization, 95
- Vanadium. National Academy of Sciences, 32
- World Review of Nutrition and Dietetics, Vol. 19. G.H. Bourne, Editor. S. Karger, 32
- World Review of Nutrition and Dietetics, Vol. 22. G.H. Bourne, Editor. S. Karger AG, 319
- BOTULISM**
neurotoxin production by *Clostridium botulinum*, necessity of bacteriophage for, 120
- BRAIN**
cellular development, following fetal thyroidectomy, 91
changes, in B-complex vitamin deficiency, 21
destruction of ventromedial hypothalamus, prevention by phlorhizin, 23
fatty acid composition, on linoleate and linolenate diets, 278
growth, in kwashiorkor, 107
human, development in fetal and postnatal periods, 6
lesions, in copper-deficient rats, 306
metabolism of pyruvate (Nutrition Classic), 211
pyridoxal phosphate in, effect of pyridoxine feeding on, 214
storage of ionic copper in, 51
- BRAN**
dietary, effect on colonic function, 70
- BRAZIL**
espichamento in, cattle disease from calcinogenic plants, SA1
- BREAD**
leavened and unleavened, availability of zinc in, 18
- CADMIUM**
poisoning, retinol excretion after, 244
- CALCIFICATION**
bone, role of silicon in, SA257
soft tissues, in S. malacoxylon poisoning, SA1
- CALCINOSIS**
in cattle from S. malacoxylon poisoning, SA1

CALCITONIN (see HORMONES)

CALCIUM

- absorption
 - in renal disease, 209
 - in S. malacoxylon poisoning, SA1
- binding, role of gamma-carboxyglutamic acid in prothrombin, 25
- relation to silicon in mitochondria, SA257
- transport, in ileum, 84

CALORIC INTAKE

- decreased, effect on thyroid function, 88
- excessive, hyperlipidemia from, 44
- relation to etiology of diabetes, SA193
- relation to growth (Nutrition Classic), 241

CANADA

- study of dietary sources of selenium in, 138

CANCER

- colon
 - relation to diet, 136
 - relation to dietary fiber, SA321
- from nitrosamines, 19

CARBOHYDRATE

- analysis (Nutrition Classic), 112
- constituents of dietary fiber, SA321
- dietary, relation to dental caries and plaque formation, SA353
- fructans, formation by dental plaque bacteria, SA353
- glucose
 - blood levels, in small-for-gestational-age infants after alanine administration, 168
 - glucoreceptor cells, effect of phlorhizin on, 23
 - tolerance, in insulin resistance, 199
- glucose tolerance factor, SA129
- glycogen, liver levels, effect of insulin and glucagon on, 347
- intake, relation to etiology of diabetes, SA193
- lactose, in milk from undernourished mothers, 42
- polysaccharides, from bacterial metabolism, role in dental plaque formation, SA353
- starch
 - hyperlipidemia from, 44
 - response of pancreatic amylase secretion to feeding, 187
- sucrose, hyperlipidemia from, 44
- xylose
 - absorption, in pernicious anemia, SA33
 - approved diagnostic agent, N320

CARBOXYLATION

- glutamyl residues, in production of prothrombin, 25
- role of biotin in, SA97

CARIES (see TEETH)

CASIMIR FUNK

- nutrition classic, 176

CATTLE

- wasting disease in, cobalt deficiency, SA65

CELIAC DISEASE

- cell-mediated immunity to gliadin in, 267

CELL

- multiplication, in human brain, 6

CELLULAR IMMUNITY

- in malnourished children, 334

CEREAL GRAINS

- bound forms of niacin in, review, SA289
- selenium in, 138
- world production, SA225

CEREBROSIDES

- brain, effect of essential fatty acid deficiency on, 278

CERULOPLASMIN

- blood levels, in steely-hair syndrome, N189
- storage in tissues, nontoxic form, 51

CHILDREN

- diarrhea in, causes of, N286
- growth of, role of somatomedins in, 262
- malnourished
 - immune deficiency in, 334
 - lymphocyte function in, 110
 - potassium metabolism in, 74
- prevention of goiter in (Nutrition Classic), 272
- Southern Tunisian, height-weight studies in, 235

CHLOROPHENYLALANINE

- production of experimental phenylketonuria by, 276

CHOLESTEROL

- bile, effect of diet on, 72
- blood levels
 - during total parenteral nutrition of low birth-weight infants, 270
 - effect of niacin on, SA289
 - effect of starch versus sucrose on, 44
 - in massive obesity, 40
 - in type III hyperlipoproteinemia, 173
 - in vegetarians versus controls, N285
- conversion to other sterols and bile acids, by intestinal flora, 136
- dietary
 - effect on bile composition, 72
 - restriction for diabetics, SA193
- synthesis
 - biliary, effect of taurocholate on, 202
 - effect of hydroxycitrate on, 53

CHOLIC ACID

- conjugates, blood levels, effect of meals on, 202

CHROMATOGRAPHY

- for separation of folic acid polyglutamates, 115

CHROMIUM (see TRACE ELEMENTS)

CITRATE

- levels in liver, in vitamin B₁₂ deficiency, 85

COAGULATION

- blood, in hypervitaminosis E, 269

COBALT (see TRACE ELEMENTS)

COENZYMES

- CoA, levels of CoA intermediates in vitamin B₁₂ deficiency, 85
- methyl tetrahydrofolate, transport form, 118
- NAD⁺ and NADH levels in liver, in vitamin B₁₂ deficiency, 118
- pyridoxal phosphate
 - in brain, in vitamin B₆ deficiency, 21
 - metabolism in liver, regulation of, 214
- vitamin B₁₂, in animal metabolism, 217

COLLAGEN

- metabolism, role of silicon in, SA257

CONNECTIVE TISSUE

- role of silicon in, SA257

CONSTIPATION

- relation to dietary fiber, SA321

COOKING

- losses of vitamins in, 123

COPPER (see TRACE ELEMENTS)

- CORN
 diet, relation to niacin requirement (Nutrition Classic), 303
- COUNCIL ON FOODS AND NUTRITION
 termination by AMA and past contributions, N29, N64
- CROCODILES
 amino acid utilization by, 281
- CROP SAC
 pigeon, lipoprotein lipase activity in, effect of prolactin on, 341
- CYCLIC AMP
 effect on folate metabolism, 57
- DEMYELINATION
 in vitamin B₁₂-depleted bats, 217
- DEOXYRIBONUCLEIC ACID
 content of brain
 after fetal thyroidectomy, 91
 index of growth, 6
 in vitamin B₆ deficiency, 21
 synthesis, in HeLa cells, effect of folic acid binding protein on, 183
- DIABETES
 prevention and therapy, review, SA193
 treatment with chromium salts, SA129
- DIARRHEA
 after jejunio-ileal bypass, 38
 in children, causes of, N286
- DIET
 cobalt content of, SA65
 control of type III hyperlipoproteinemia by, 173
 corn, relation to niacin requirement (Nutrition Classic), 303
 fat, influence on body fat composition in infants, 236
 fiber in, effect on colonic function, 70, SA321
 histidine in, effect on nitrogen balance, 200
 infants, deficient in vitamin E, SA161
 linoleate and linolenate in, effect on fatty acid composition of brain, 278
 millet, effect on niacin requirement, review, SA289
 relation to bile composition, 72
 relation to diabetes, SA193
 relation to excretion of sterols and bile acids, 136
 sources of selenium, 138
 starch versus sucrose, hyperlipidemia from, 44
- DIGESTION
 triglycerides, by esophageal aspirates, 205
- DIHYDROXYCHOLECALCIFEROL (see VITAMIN D)
- DISEASE
 α-B-lipoproteinemia, vitamin E deficiency in, 206
 alpha-1-antitrypsin deficiency, N189
 celiac, cell-mediated immunity to gliadin in, 267
 chronic myelogenous leukemia, folate binder in serum and leukocytes in, 9
 congestive heart failure, taurine level in heart muscle in, 343
 cystic fibrosis, vitamin E deficiency in, SA161
 endemic goiter, relation to antithyroid agents, 171
 intestinal, relation to dietary fiber, SA321
 sickle cell, zinc deficiency in, 266
 tropical sprue, small intestine changes in, SA33
 type III hyperlipoproteinemia, 173
 viral hepatitis A, new diagnostic tests for, 331
- DNA/WEIGHT RATIOS
 of organs, after fetal thyroidectomy, 91
- DRUGS
 anticonvulsant, effect on calcium metabolism, 209, N221
 antithyroid agents, relation to endemic goiter, 171
 clofibrate, effects in hypervitaminosis E, 269
 diiodohydroxyquin, treatment of acrodermatitis enteropathica, 327
 methotrexate
 binding by macromolecular factor in serum and leukocytes, 9
 transport in vitamin B₁₂ deficiency, 118
 niacin, pharmacologic effects, review, SA289
 nitrosamines, cancer from, 19
 phlorhizin, effect on transport mechanisms in glucoreceptor cells, 23
 warfarin, effects in hypervitaminosis E, 269
- DRYING
 foods, nutrient losses during, 123
- EDEMA
 in premature infants with vitamin E deficiency, SA161
- ELASTIN
 synthesis, role of copper in, 306
- ELECTROENCEPHALOGRAM
 changes, in B-complex vitamin deficiency, 21
- ELECTROLYTE
 changes, in malnutrition, 74
- ENERGY
 availability, from dietary fiber, SA321
 requirements for man, from FAO/WHO handbook, 147
- ENRICHMENT
 foods, with iron, problems in, 46
- ENTEQUE SECO
 cattle disease in Argentina, from calcinogenic plants, SA1
- ENTEROTOXINS
 cause of diarrhea in children, N286
- ENZOOTIC CALCINOSIS
 cattle disease in Germany, from calcinogenic plants, SA1
- ENZYMES
 acetyl-CoA carboxylase, role of biotin in, SA97
 ALA dehydratase, in liver, in vitamin E deficiency, SA161
 ALA synthetase, in bone marrow, in vitamin E deficiency, SA161
 alkaline phosphatase
 blood levels, after jejunio-ileal bypass, 38
 in liver and bone, during anticonvulsant drug therapy, 209, N221
 amylase, pancreatic, response to oral or parenteral carbohydrate feeding, 187
 arginase, in liver, changes on low protein intake, 180
 argininosuccinate lyase, in liver, changes on low protein intake, 180
 argininosuccinate synthetase, in liver, changes on low protein intake, 180
 ATP-citrate lyase, effect of hydroxycitrate on, 53
 beta-glucuronidase, in feces, measure of intestinal microbial activity, 136
 carnitine acyltransferase, role in regulation of ketogenesis, 347
 cysteine sulfinic acid decarboxylase, activity in vitamin B₆ deficiency, 343

- cytochrome oxidase, in brain, in copper deficiency, 306
 - dihydrofolate reductase, in liver, in vitamin B₁₂ deficiency, 118
 - disaccharidase, in intestinal mucosa, in pernicious anemia, SA33
 - formiminotransferase, effect of hormones on, 57
 - glucosyltransferases, bacterial, role in dental plaque formation, SA353
 - glutamate dehydrogenase, in liver, changes on low protein intake, 180
 - glutathione peroxidase, activity in vitamin E deficiency, SA161
 - hydroxylating, in vitamin E deficiency, SA161
 - lipase
 - in esophageal aspirates, 205
 - pancreatic, response to fat feeding, 187
 - secretion, dependence on caloric content of meal, 169
 - lipoprotein lipase, effect of prolactin on activity 341
 - methyl tetrahydrofolate-homocysteine transmethylase activity in vitamin B₁₂ deficiency, 118
 - methylene tetrahydrofolate reductase, inhibition by S-adenosyl methionine, 118
 - phenylalanine hydroxylase, inhibition by chlorophenylalanine, 276
 - phosphatases, inhibition of, effect on pyridoxal phosphate levels in hepatocytes, 214
 - phytate-splitting, in yeast, 18
 - proteolytic, pancreatic, response to protein feeding, 187
 - pyridoxal phosphate dependent, in brain, in vitamin B₆ deficiency, 21
 - pyridoxine phosphate oxidase, role in pyridoxal phosphate metabolism, 214
 - pyruvate carboxylase, role of biotin in, SA97
 - sialyltransferase, in alpha-1-antitrypsin deficiency disease, N189
 - sulfite oxidase, molybdenum in, 185
 - superoxide dismutase, in brain, in copper deficiency, 306
 - transaminase
 - blood levels, after jejuno-ileal bypass, 38
 - in liver, changes on low protein intake, 180
 - trypsin, secretion, dependence on caloric content of meal, 169
 - xanthine oxidase
 - in vitamin E deficiency, 59, SA161
 - molybdenum in, 185
- EPINEPHRINE** (see HORMONES)
- ERYTHROCYTE**
- sickle cells, relation to zinc deficiency, 266
 - stability, role of vitamin E in, 206, SA161
- ERYTHROPOIESIS**
- regulation by iron, 144
- ESOPHAGUS**
- aspirates, lipase activity in, 205
- ESPICHAMENTO**
- cattle disease in Brazil, from calcinogenic plants, SA1
- ETHIOPIA**
- study of infant brain growth in, 107
- EYE**
- pathology, in diabetes, SA193
 - retinal function, role of taurine in, 343
- FAO/WHO**
- recommended intakes of nutrients, 147
- FAT**
- absorption, defect in, vitamin E deficiency in, SA161
 - blood levels, elevated, in iron deficiency anemia, 55
 - body, composition of infants, influence of dietary fat, 236
 - content of brain, after fetal thyroidectomy, 91
 - dietary
 - influence on composition of body fat of infants, 236
 - relation to bile composition, 72
 - response of pancreatic lipase to, 187
 - excretion, in pernicious anemia, SA33
 - fat-free parenteral feeding, essential fatty acid deficiency from, 329
 - hyperlipidemia, from starch versus sucrose, 44
 - in milk from undernourished mothers, 42
 - intake, relation to etiology of diabetes, SA193
 - liver, levels after bypass operation, 78
 - malabsorption, vitamin E deficiency in, SA161
 - plasma lipids, association with vitamin E, SA161
 - subcutaneous, in infants, measurement by skinfold thickness, 7
 - synthesis, effect of hydroxycitrate on, 53
 - triglycerides
 - blood, composition after fat-free parenteral feeding, 329
 - blood levels
 - effect of starch versus sucrose on, 44
 - in insulin resistance, 199
 - in type III hyperlipoproteinemia, 173
 - in vegetarians versus controls, N285
 - digestion by esophageal aspirates, 205
 - utilization, effect of prolactin on, 341
- FATTY ACIDS**
- arachidonic
 - in brain lipids, in essential fatty acid deficiency, 278
 - tissue levels in vitamin E deficiency, SA161
 - autoxidation from supplemental iron in foods, 46
 - biosynthesis, role of biotin in, SA97
 - essential
 - deficiency
 - composition of brain lipids in, 278
 - from constant infusion of fat-free nutrients, 329
 - linoleate and linolenate, dietary, effect on fatty acid composition of brain, 278
 - oxidation, control by insulin and glucagon, 347
 - plasma levels, effect of insulin and glucagon on, 347
 - unsaturated, dietary, effect on body fat composition of infants, 236
- FECES**
- water content, relation to dietary fiber, SA321
- FEDERAL TRADE COMMISSION**
- proposed regulations on food advertising, N29, 246
- FERRITIN**
- serum levels, relation to body iron stores, 11
- FERTILIZERS**
- significance in world food production, SA225
- FIBER**
- in diet, effect on colonic function, 70, SA321
- FISH**
- selenium in, 138

FLOUR

effects of added iron salts on, 46

FLUORIDE (see TRACE ELEMENTS)

FLUOROSIS

genu valgum in, 76

FOLIC ACID

blood levels, in maternal and newborn, 298

deficiency

central nervous system abnormalities in, 21

intestinal absorption in, SA33

length of glutamyl side chain, in bacteria and liver, 115

macromolecular binder, in serum and leukocytes, 9

metabolism

effect of hormones on, 57

in vitamin B₁₂ deficiency, 118

supplementation, effect on anemia in pregnancy, 332

uptake by HeLa cells, inhibition by folic acid

binding protein, 183

FOOD

additives, hyperkinetic effects of, study of, N220

advertising, proposed FTC regulations, N29, 246

availability of chromium in, SA129

cobalt content of, SA65

consumption, effect of hydroxycitrate on, 53

fortification, with iron, problems in, 46

intake, effect of phlorhizin on hypothalamic

glucoreceptor cells, 23

iron in, absorption of, 238

needs, World Food Conference, SA225

poisoning, from Clostridium types, 120

processing, effects on nutritional values, 123

FOOD AND AGRICULTURE ORGANIZATION

Food and Nutrition, new periodical, N255

FOOD AND DRUG ADMINISTRATION

proposals for regulating vitamin and mineral

products, N220

FOOD AND NUTRITION

new FAO periodical, N255

FOOD AND NUTRITION BOARD

special report on hazards of overuse of vitamin D, 61

FOOD TECHNOLOGY

World Food Conference, SA225

FORTIFICATION

foods, with iron, problems in, 46

FUNGUS

infections, in malnourished children, 334

GALLSTONES

relation to dietary fats, 72

GAMMA-AMINOBUTYRIC ACID

synthesis, in brain, in vitamin B₆ deficiency, 21

GAMMA-CARBOXYGLUTAMIC ACID

new amino acid in prothrombin, 25

GENETICS

alkaptonuria (Nutrition Classic), 81

in etiology of diabetes, SA193

role in establishing norms of growth in children, 235

GENU VALGUM

due to fluoride toxicity, 76

GHANA

study of malnutrition and immune deficiency, 334

GLIADIN (see PROTEIN)

GLUCAGON (see HORMONES)

GLUCONEOGENESIS

defect, in samll-for-gestational-age infants, 168

GLUCOSE (see CARBOHYDRATE)

GLUCOSE TOLERANCE FACTOR

review, SA129

GLUTAMYL

side chains in folic acid, in bacteria and liver, 115

GLYCOGEN (see CARBOHYDRATES)

GLYCOLYSIS

in dental plaque bacteria, carbohydrate sources for, SA353

GOITER

endemic, relation to antithyroid agents, 171

prevention (Nutrition Classic), 272

structure of thyroid gland in (Nutrition Classic), 338

GOLDSMITH, GRACE A.

memorial tribute, N314

GOLD THIOGLUCOSE

competition with phlorhizin, for transport mechanisms in glucoreceptor cells, 23

GROWTH

bacteria, in dental plaques, energy sources for, SA353

brain

in fetal and postnatal periods, 6

in kwashiorkor, 107

crocodile, protein synthesis during, 281

during caloric restriction (Nutrition Classic), 241

low birthweight infants, on total parenteral

nutrition, 270

norms of children in underdeveloped nations, 235

role of silicon in, SA257

role of somatomedins in, 262

HAIR

copper in, after feeding excessive copper, 51

HARPENDEN CALIPERS

for measuring subcutaneous fat and interstitial water in infants, 7

HAWAII

naahelus disease in, cattle disease from

calcinogenic plants, SA1

HEAD

circumference, changes in kwashiorkor, 107

HEARING

disability, in uremia, N287

HEART

copper in, after feeding excessive copper, 51

disease, in type III hyperlipoproteinemia, 173

incorporation of dietary selenium in, 138

lesions, in copper-deficient rats, 306

muscle, role of taurine in, 343

silicon content, SA257

toxic effects of cobalt on, SA65

HEIGHT-WEIGHT

measurements, of children in Southern Tunisia, 235, N350

HELA CELLS

uptake of folate by, effect of folic acid binding protein on, 183

HEMATOCRIT

values, statistical studies, N190

HEME

- iron, absorption of, 238
- synthesis, in vitamin E deficiency, SA161

HEMOCHROMATOSIS

- serum ferritin levels in, 11

HEMOGLOBIN

- levels
 - in massive obesity, 40
 - in pregnancy, effect of iron supplementation on, 332
 - relation of vitamin E and iron intake on, 206

HISTOLOGY

- spinal cord, in vitamin B₁₂-depleted rats, 217

HOLLAND

- body fat composition of infants, relation to dietary fat, 236

HOMOGENITIC ACID

- excretion (Nutrition Classic), 81

HORMONES

- ACTH, plasma levels, in adults with protein-calorie malnutrition, 299
- calcitonin, secretion in *S. malacoxylon* poisoning, SA1
- cortisol, plasma levels, in adults with protein-calorie malnutrition, 299
- epinephrine, effect on formiminotransferase activity, 57
- glucagon
 - blood levels in small-for-gestational-age infants after alanine administration, 168
 - effect on formiminotransferase activity, 57
 - effect on ketogenesis, 347
 - role in hypertriglyceridemia, 199
- growth
 - plasma levels, in adults with protein-calorie malnutrition, 299
 - somatomedins, 262
- hypothalamic thyroid-releasing, secretion in undernutrition, 88
- insulin
 - blood levels in small-for-gestational-age infants after alanine administration, 168
 - effect on disappearance of blood amino acids in crocodiles, 281
 - effect on formiminotransferase activity, 57
 - effect on ketogenesis, 347
 - reaction with receptors, role of glucose tolerance factor in, SA129
 - resistance, in obesity, SA193
 - role in hypertriglyceridemia, 199
- norepinephrine, in brain, in copper deficiency, 306
- prolactin, effect on lipoprotein lipase activity, 341
- thyroid
 - in maternal and fetal blood, after fetal thyroidectomy, 91
 - plasma levels, in adults with protein-calorie malnutrition, 299
 - role of cobalt in synthesis, SA65
- thyrotropin, plasma levels in undernourished newborn rats, 88

HYDROGEN

- production, from colonic bacteria, SA321

HYDROXYCITRATE

- effect on lipogenesis, appetite and body weight, 53

HYPERKINESIS

- from food additives, study of, N220

HYPERLIPIDEMIA

- from starch versus sucrose, 44

HYPERPHAGIA

- production by phlorhizin injection into hypothalamus, 23

HYPERTENSION

- heart muscle taurine concentration in, 343

HYPERTRIGLYCERIDEMIA

- endogenous, in cellular insulin resistance, 199

HYPOGLYCEMIA

- in small-for-gestational-age infants, 168

HYPOTHALAMUS

- glucoreceptor cells, transport mechanisms in, effect of phlorizin on, 23

IMMUNITY

- deficiency, in malnourished children, 110, 334
- to gliadin, in celiac disease, 267

IMMUNOASSAY

- serum ferritin, 11

IMMUNOGLOBULINS

- levels, in malnourished children, 334

INDIA

- field trials on effect of iron supplementation on gestational anemia in, 332
- genu valgum associated with fluorosis, 76

INFANTS

- acrodermatitis enteropathica in, 327
- anemia in, iron deficiency as cause of, 144
- body fat composition of, 7, 236
- brain development in, 6
- from iron-deficient mothers, hyperlipemia in, 55
- hazards of overuse of vitamin D, special report, 61
- kwashiorkor in, brain growth in, 107
- low birthweight, use of total parenteral nutrition for, 270
- malnourished, lymphocyte function in, 110
- premature
 - renal handling of salt by, 105
 - vitamin E therapy in, SA161, 206
- small-for-gestational-age, defective gluconeogenesis in, 168

INFECTION

- relation to iron status, 103
- resistance to, in malnourished children, 110, 334

INFLAMMATION

- anemia, role of reticuloendothelial cells in, 213
- serum ferritin levels and iron storage in, 11

INSULIN (see HORMONES)

INTESTINE

- absorption
 - amino acids, by the crocodile, 281
 - calcium
 - in renal disease, 209
 - in *S. malacoxylon* poisoning, SA1
 - in the ileum, 84
 - chromium salts, SA129
 - cobalt, SA65
 - copper, in steely-hair syndrome, N189
 - iron
 - in inflammatory diseases, 213
 - studies with isotopic iron, 238
 - phosphorus, in *S. malacoxylon* poisoning, SA1
 - silicon, SA257
 - studies on, D-xylose approved as diagnostic agent, N320
 - α -tocopherol polyethylene glycol succinate, 206
 - xylose, in pernicious anemia, SA33

- zinc
 - from wholemeal breads, 18
 - in acrodermatitis enteropathica, 327
 - bacteria and viruses in, cause of diarrhea in children, N286
 - bypass operation, liver changes after, 38, 78
 - colon
 - bacteria in, relation to dietary fiber, SA321
 - cancer of, relation to diet, 136
 - effect of dietary fiber on, 70
 - copper in, after feeding excessive copper, 51
 - fat synthesis in, effect of hydroxycitrate on, 53
 - flora, effect on sterol and bile acid excretion, 136
 - formiminotransferase in, effect of hormones on, 57
 - function, effect of dietary fiber on, SA321
 - ileal resection, effect on cholic acid conjugates blood levels, 202
 - malabsorption, vitamin E deficiency in, SA161
 - mucosa, cell-mediated immunity to gliadin in, in celiac disease, 267
 - short bowel syndrome, 169
 - villi, changes in pernicious anemia, SA33
- IODINE (see TRACE ELEMENTS)**
- IRAN**
- zinc deficiency and use of unleavened bread, 18
- IRON**
- absorption
 - in inflammatory diseases, 213
 - studies with isotopic iron, 238
 - body storage, relation to serum ferritin levels, 11
 - deficiency
 - effect on liver iron content, 55
 - infection in, 103
 - deficiency anemia, in pregnancy, effect of iron supplementation on, 332
 - dietary
 - relation to etiology of diabetes, SA193
 - relation to hyperlipemia, 55
 - fortification of foods, problems in, 46
 - metabolism, effect of cobalt on, SA65
 - regulation of erythropoiesis by, 144
 - requirements for man, from FAO/WHO handbook, 147
 - role in vitamin E metabolism, 206
 - serum levels
 - in histidine deficiency, 200
 - in inflammatory diseases, 213
 - tissue levels, after feeding excessive copper, 51
- ISOTOPE**
- labeling of iron, use in iron absorption studies, 238
- JAMAICA**
- Manchester wasting disease in, cattle disease from calcinogenic plants, SA1
- JAPAN**
- changes in incidence of diabetes in, SA193
- JOWAR**
- diet, effect on niacin requirement, review, SA289
- KETOGENESIS**
- control by insulin and glucagon, 347
- KIDNEY**
- copper in, after feeding excessive copper, 51
- disease
 - calcium absorption in, 209
 - chronic dialysis for, neuropathy in, N287
 - relation to somatomedin levels, 262
 - failure, serum ferritin levels and iron storage in, 11
 - glutathione peroxidase in, in selenium and vitamin E deficiency, SA161
 - handling of salt, by preterm infants, 105
 - incorporation of dietary selenium in, 138
 - nephrosis, in massive obesity, 40
 - pathology, in diabetes, SA193
 - weight, effect of undernutrition in newborn rats, 88
- KNOCK KNEE**
- due to fluoride toxicity, 76
- KWASHIORKOR**
- brain growth in, 107
 - immunity response in, 110, 334
 - in adults, endocrine changes in, 299
 - thyroid function in, 88
- LACTATE**
- production in dental plaques, SA353
- LACTATION**
- in undernourished women, 42
 - iron deficiency in, effect on offspring, 55
 - lipoprotein lipase activity in mammary gland, 341
- LETTERS TO THE EDITOR**
- Lowenstein, F. W., 350
 - Luyken, R., 191
 - Rasmussen, H., 31
- LEUKOCYTES**
- folate binder in, 9
 - response, in celiac disease, 267
- LIFE SPAN**
- relation to caloric intake (Nutrition Classic), 241
- LIGNIN**
- constituent of dietary fiber, SA321
- LIPOPROTEIN**
- α - β -lipoproteinemia, vitamin E deficiency in, 206
 - blood levels
 - effect of niacin on, SA289
 - in type III hyperlipoproteinemia, 173
 - in vegetarians versus controls, N285
 - pattern, in offspring of iron-deficient mothers, 55
 - synthesis in liver, in cellular insulin resistance, 199
 - type III hyperlipoproteinemia, pathologic changes in, 173
- LIVER**
- alkaline phosphatase in, during anticonvulsant drug therapy, 209, N221
 - alpha-1-antitrypsin deficiency, N189
 - aminolevulinic acid dehydratase in, in vitamin E deficiency, SA161
 - cancer, from nitrosamines, 19
 - disease, serum ferritin levels and iron storage in, 11
 - enzymes, changes on low protein intake, 180
 - failure, after jejunio-ileal bypass, 38
 - fat
 - after bypass operation, 78
 - synthesis, effect of hydroxycitrate on, 53
 - folates, in vitamin B₁₂ deficiency, 118
 - formiminotransferase in, effect of hormones on, 57
 - function, bile acid excretion test for, N285

- glucogen, levels, effect of insulin and glucagon on, 347
- glutathione peroxidase in, in selenium and vitamin E deficiency, SA161
- incorporation of dietary selenium in, 138
- iron storage in, in iron deficiency, 55
- lecithin synthesis in, role of bile salts in, 202
- length of folic acid glutamyl side chains in, 115
- level of CoA intermediates in, in vitamin B₁₂ deficiency, 85
- lipoprotein production by, in cellular insulin resistance, 199
- pyridoxal phosphate metabolism in, regulation of, 214
- release of iron, in inflammatory diseases, 213
- silicon content, SA257
- storage of ionic copper in, 51
- taurine metabolism in, 343
- viral hepatitis A, new diagnostic tests for, 331
- xanthine oxidase, in vitamin E deficiency, 59, SA161
- LUNG**
- alpha-1-antitrypsin deficiency, N189
- copper in, after feeding excessive copper, 51
- glutathione peroxidase in, in selenium and vitamin E deficiency, SA161
- incorporation of dietary selenium in, 138
- silicon content, SA257
- volumes, in massive obesity, 40
- LYMPHOCYTE**
- function
- in iron deficiency, 103
- in malnutrition, 110
- MAGNESIUM**
- requirements for man, from FAO/WHO handbook, 147
- MALABSORPTION**
- D-xylose approved as a diagnostic agent, N320
- syndromes, use of hydroxycholecalciferol in, 209
- MALARIA**
- incidence, relation to iron status, 103
- MALATE**
- levels in liver, in vitamin B₁₂ deficiency, 85
- MALNUTRITION**
- after jejuno-ileal bypass, 38
- brain development in, 6, 21, 107
- composition of mother's milk in, 42
- in children
- immune deficiency in, 334
- in etiology of diabetes, SA193
- lymphocyte function in, 110
- potassium retention in, 74
- protein-calorie, in adults, endocrine changes in, 299
- salivary secretion in, 178
- thyroid function in, 88
- MAMMARY GLAND**
- lipoprotein lipase activity in, effect of prolactin on, 341
- MANCHESTER WASTING DISEASE**
- cattle disease in Jamaica, from calcinogenic plants, SA1
- MANGANESE** (see TRACE ELEMENTS)
- MARASMUS**
- immune deficiency in, 334
- in adults, endocrine changes in, 299
- thyroid function in, 88
- MATERNAL NUTRITION**
- comparison of maternal and newborn blood levels of vitamins, 298
- MEAT**
- cobalt in, SA65
- curing, nitrosamines from, 19
- iron, absorption of, 238
- selenium in, 138
- MEGAVITAMIN**
- therapy, review, SA289
- MEMBRANES**
- role of vitamin E in, 206
- MENKES SYNDROME**
- copper metabolism in, N189
- METHYLMALONYL CoA**
- levels in liver, in vitamin B₁₂ deficiency, 85
- METHYL TRAP**
- theory of secondary folate deficiency in vitamin B₁₂ deficiency, 118
- MICROSOMES**
- liver
- destruction of vitamin D metabolites by, 209, N221
- lipid synthesis in, 202
- MILK**
- cobalt in, SA65
- composition, in undernourished women, 42
- fatty acid composition, effect of essential fatty acid deficiency on, 278
- formula, with potassium, in correcting electrolyte changes in rehabilitation of malnourished children, 74
- human, suitability for preterm infants, 105
- production, effect of prolactin on, 341
- selenium in, 138
- vitamins in, relation to mother's diet, N191
- MILLET**
- diet, effect on niacin requirement, review, SA289
- MINERALS**
- products, FDA proposals for regulation of, N220
- MITOCHONDRIA**
- silicon in, SA257
- MOLYBDENUM** (see TRACE ELEMENTS)
- MOTILITY**
- colon, effect of dietary fiber on, 70
- MUCOPOLYSACCHARIDE**
- metabolism, role of silicon in, SA257
- MUSCLE**
- damage, in vitamin E deficiency, SA161
- fatty acid composition of, effect of essential fatty acid deficiency on, 278
- glutathione peroxidase in, in selenium and vitamin E deficiency, SA161
- histidine in, in histidine deficiency, 200
- potassium in, in malnourished children, 74
- silicon content, SA257
- taurine metabolism in, 343
- weight, effect of undernutrition in newborn rats, 88
- MYELINATION**
- changes, in copper-deficient rats, 306
- in malnutrition, 6
- NAAHELU DISEASE**
- cattle disease in Hawaii, from calcinogenic plants, SA1
- NATIONAL ACADEMY OF SCIENCES**
- special report on hazards of overuse of vitamin D, 61
- NECROSIS**
- ventromedial hypothalamus, after gold thioglucose injection, 23.

NERVE

- damage, in vitamin B₁₂-deficient bats, 217
- destruction of ventromedial hypothalamus, prevention by phlorhizin, 23
- neuropathy, in renal patients on chronic dialysis, N287

NEUROCHEMISTRY

- changes, in vitamin B₆ deficiency, 21

NIACIN

- association with chromium, in glucose tolerance factor, SA129
- deficiency, central nervous system abnormalities in, 21
- niacinamide, blood levels, in maternal and newborn, 298
- requirement, in man (Nutrition Classic), 303
- review, SA289

NITRITES

- role in cancer production by nitrosamines, 19

NITROGEN

- balance
 - effect of histidine deficiency in, 200
 - in adults with protein-calorie malnutrition, 299
- metabolism, changes with protein intake, 180

NITROSAMINES

- production of cancer by, 19

N-METHYLNICOTINAMIDE

- excretion, measure of niacin nutriture, SA289

N-METHYL-2-PYRIDONE-5-CARBOXYLAMIDE

- excretion, measure of niacin nutriture, SA289

NOREPINEPHRINE (see HORMONES)

NOTES

- Alpha-1-antitrypsin deficiency-liver disease, 189
- Alpha omega alpha interviews
 - Coggeshall, L.T., 317
 - Corner, G.W., 317
 - Cummings, M.M., 317
 - Dragstedt, L.R., 317
 - Enders, J.F., 317
 - Engel, G.L., 317
 - Genest, J., 317
 - Goldsmith, G.A., 317
 - Harrison, T.R., 317
 - Hastings, A.B., 317
 - Holman, E., 317
 - Huggins, C.B., 317
 - McDermott, W., 317
 - Meyer, K.F., 317
 - Rigler, L.G., 317
 - Taussig, H.B., 317
 - Taylor, H.C., Jr., 318
 - Walker, M., 318
 - Wangensteen, O.W., 318
 - Warren, S., 318
 - Watson, C.J., 318
 - Wearn, J.T., 318
 - Wintrobe, M.W., 318
 - Wood, W.B., Jr., 318
- Anticonvulsant drugs and calcium metabolism, 221
- Bile acid excretion—a new test of functioning liver mass, 285
- Committee on nutritional anthropology, 318
- "Conquest of Famine" receives Nutrition Foundations' book award, 127
- Cooper metabolism in the steely-hair syndrome, 189
- Current status of AMA Council on Foods and Nutrition, 64

D-Xylose approved as a diagnostic agent, 320

FDA proposals regarding vitamin and mineral products, 220

FDA toxicology advisory committee formed, 351

Fellowships in clinical nutrition, 127

Fellowships in clinical nutrition, 288

FTC proposed trade regulation rule on food advertising, 30

Grace A. Goldsmith, M.D., 314

Leaders in American medicine, 316

Meeting Announcements

American Diabetes Association

Diabetes in review: clinical conference, 1976, 288

Eighth allied health postgraduate course in diabetes, 288

Perspectives in current diabetes research, first combined health care professional course in diabetes, 256

36th annual meeting of American Diabetes Association, 288

Council on epidemiology and prevention, international society of cardiology, 30

89th annual meeting of the association of official analytical chemists, 160

International organization for the study of human development, 96

Nutrition in mothers, infants and preschool children, 30

Sixth Argentine Congress of nutrition, 351

Symposium on fat emulsions in parenteral nutrition, 160

Symposium on the myelomeningocele patient—a multidisciplinary approach, 318

Symposium on nutrition and drug interrelations, 223

Symposium on nutritional disorders of American women, 160

Symposium on specifications for food chemicals, 95

Tenth international congress of nutrition, 63

National advisory committee on hyperkinesis and food additives, 220

Neuropathy in renal patients on chronic dialysis, 287

New FAO periodical, 255

New fellowships in nutrition available for physicians, 159

New NAS/NCR report on program planning, 319

New publications

Baroda Journal of Nutrition, 32

Hematocrit values of youths 12 to 17 years, United States, 190

Plasma lipid and lipoproteins in vegetarians and controls, 285

Recent awards in nutrition, 255

Severe diarrhea in children—new ideas in an unsolved arena, 286

Termination of AMA Council on Foods and Nutrition, 29

NUTRITION

survey

- for study of etiology of diabetes, SA193
- use of serum ferritin levels in, 11

NUTRITION CLASSICS

Folin: "A theory of protein metabolism," 141

Funk: "The etiology of the deficiency diseases," 176

Garrod: "The incidence of alkaptonuria: a study in chemical individuality," 81

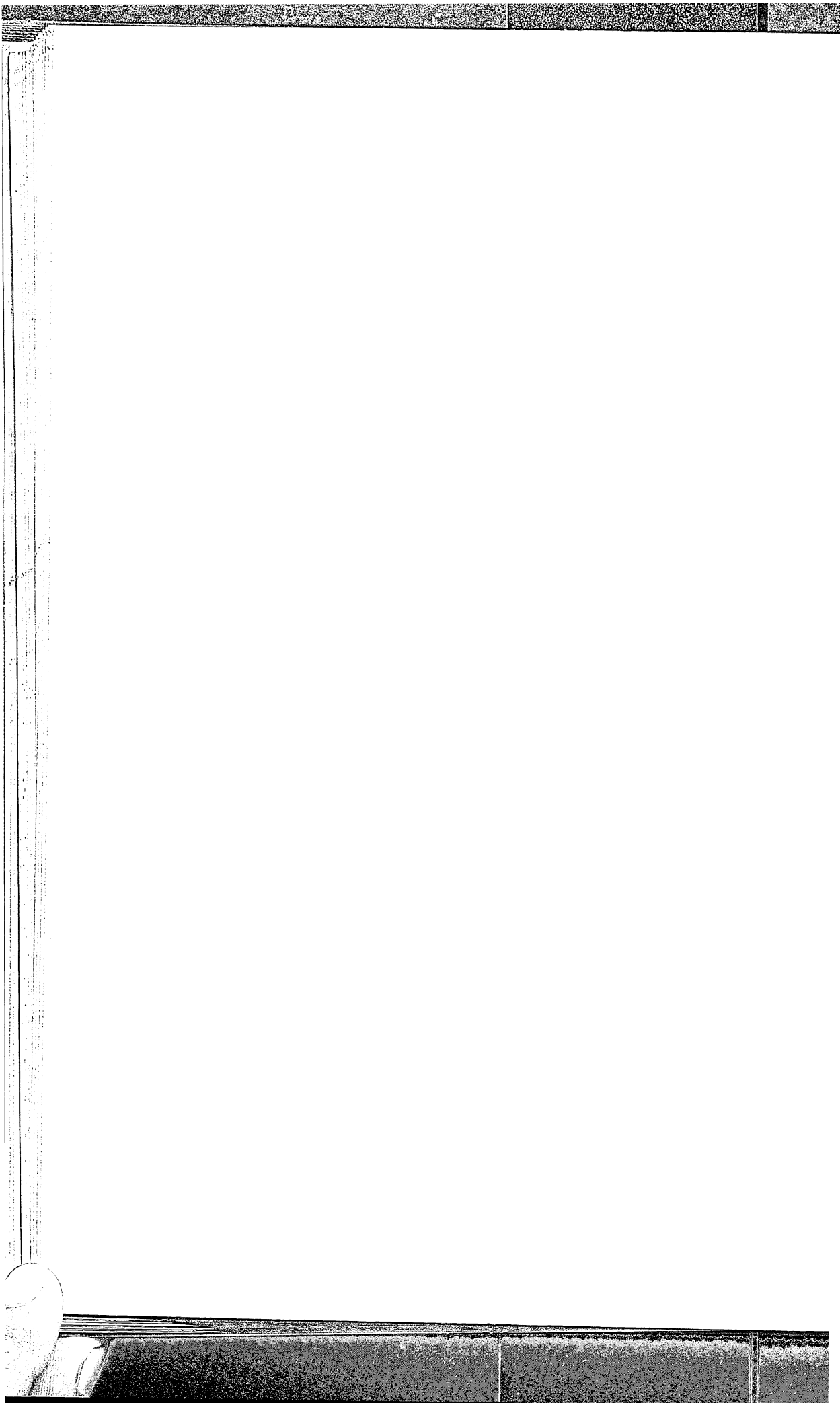
Goldsmith, Rosenthal, Gibbens and Unglaub: "Studies of niacin requirement in man," 303

- McCay, Maynard, Sperling and Barnes: "Retarded growth, life span, ultimate body size and age changes in the albino rat after feeding diets restricted in calories," 241
- McCollum, Simmonds and Becker: "Studies on experimental rickets," 48
- Marine and Kimball: "The prevention of simple goiter in man," 272
- Marine and Williams: "The relation of iodine to the structure of the thyroid gland," 338
- Peters and Thompson: "Pyruvic acid as an intermediary metabolite in the brain tissue of avitaminous and normal pigeons," 211
- Prout: "On the ultimate composition of simple alimentary substances; with some preliminary remarks on the analysis of organized bodies in general," 112
- Wilcock and Gowland Hopkins: "The importance of individual amino-acids in metabolism," 15
- NUTRITION EDUCATION**
World Food Conference, SA225
- NUTRITION FOUNDATION**
resource for exchange of personal nutrition collections, 312
- NUTRITIONAL ANTHROPOLOGY**
committee on, N318
- OAT GRASS**
toxicity and vitamin D-like substances in, SA1
- OBESITY**
in etiology of diabetes, SA193
jejuno-ileal bypass, liver changes after, 38, 78
nephrosis in, 40
- ORAL CONTRACEPTIVES**
folate binder in serum and leukocytes after, 9
- OSTEOSCLEROSIS**
in fluorosis, 76
- OXIDATION**
fatty acids, regulation by carnitine acyltransferase, 347
- PAKISTAN**
study of mother's milk in, 42
- PANCREAS**
enzyme secretions, after oral or parenteral feeding, 187
secretion, dependence on caloric content of meal, 169
- PANTOTHENIC ACID**
blood levels, in maternal and newborn, 298
- PARENTERAL FEEDING**
pancreatic enzyme secretions after, 187
with fat-free nutrients, essential fatty acid deficiency from, 329
- PARENTERAL NUTRITION**
total, for low birthweight infants, 270
- PATHOLOGY**
acrodermatitis enteropathica, 327
diabetes, SA193
enteque seco, in cattle, SA1
erythrocyte, in vitamin E deficiency, 206
fluoride toxicity, 76
hypothalamus, after phlorhizin injection, 23
kidney, in massive obesity, 40
liver, after jejuno-ileal bypass, 38
nerve, in vitamin B₁₂-depleted rats, 217
small intestine, in pernicious anemia, SA33
type III hyperlipoproteinemia, 173
- PELLAGRA**
conquest of, memorabilia of William Henry Sebrell, 310
description and history, review, SA289
etiology (Nutrition Classic), 176
- PEPTIDE**
from prothrombin, chemistry of, 25
- PERNICIOUS ANEMIA** (see ANEMIA)
- PEROXIDATION**
membrane lipids, in vitamin E deficiency, SA161
- PHENYLKETONURIA**
experimental, excretion of phenylalanine metabolites in, 276
- PHENYLACTATE**
excretion, in experimental phenylketonuria, 276
- PHENYLPYRUVATE**
excretion, in experimental phenylketonuria, 276
- PHLORHIZIN** (see DRUGS)
- PHOSPHOLIPID**
in bile, effect of diet on, 72
lecithin, synthesis in liver, role of bile salts in, 202
phosphatidylethanolamine, in brain, composition on linoleate and linolenate diets, 278
synthesis, biliary, effect of taurocholate on, 202
- PHOSPHORUS**
absorption, in S. malacoxylon poisoning, SA1
- PHYTATES**
in wholemeal bread, effect on zinc availability, 18
- PIGEON**
crop sac, lipoprotein lipase activity in, effect of prolactin on, 341
- PLACENTA**
transport of glucose tolerance factor, SA129
- PNEUMONIA**
in malnourished children, 334
- POTASSIUM**
blood levels, after jejuno-ileal bypass, 38
changes, in malnutrition, 74
dietary, relation to taurine levels in heart, 343
- POTATO**
mashed, effects of added iron salts on, 46
- POULTRY**
selenium in, 138
- PREGNANCY**
anemia in, effect of iron supplementation on, 332
development of fetal brain during, 6
folate binder in serum and leukocytes in, 9
iron deficiency in, effect on offspring, 55
- PROPIONATE**
metabolism, in vitamin B₁₂ deficiency, 85, 217
- PROPIONYL CoA**
levels in liver, in vitamin B₁₂ deficiency, 85
- PROTEIN** (see also IMMUNOGLOBULINS)
albumin
blood levels after jejuno-ileal bypass, 38
excretion, in massive obesity, 40
binding of pyridoxal phosphate by, 214
changes, during food processing and preservation, 123
content of brain, after fetal thyroidectomy, 91
content of saliva, in protein deficiency, 178
deficiency
brain growth in, 107
in adults, endocrine changes in, 299
salivary secretion in, 178

- dietary, response of pancreatic proteases to, 187
- excretion, in massive obesity, 40
- folic acid binding, effect on folate uptake by HeLa cells, 183
- gliadin, immunity to, in celiac disease, 267
- in milk from undernourished mothers, 42
- intake
 - relation to potassium retention in malnourished children, 74
 - relation to urea excretion, 180
 - role in growth of children, 235
- metabolism (Nutrition Classic), 141
- requirements for man, from FAO/WHO handbook, 147
- retinol bound compound in urine, 244
- synthesis
 - in the crocodile, 281
 - in vitamin E deficiency, SA161
- zein, inadequacy (Nutrition Classic), 15
- PROTEIN-CALORIE MALNUTRITION**
 - effect on composition of mother's milk, 42
 - in adults, endocrine changes in, 299
- PROTEIN/DNA RATIOS**
 - of organs, after fetal thyroidectomy, 91
- PROTEINURIA**
 - in massive obesity, 40
- PROTHROMBIN**
 - carboxylation of glutamyl residues in production of, 25
 - levels, effect of excess vitamin E on, 269
 - time, after jejuno-ileal bypass, 38
- PSYCHOLOGY**
 - psychosocial changes after intestinal bypass for obesity, 78
- PUBLIC HEALTH**
 - use of iron supplementation in pregnancy anemia, 332
- PYRIDOXAL** (see VITAMIN B₆)
- PYRIDOXINE** (see VITAMIN B₆)
- PYRUVATE**
 - metabolism
 - in dental plaque bacteria, SA353
 - in thiamine deficiency (Nutrition Classic), 211
- RACIAL DIFFERENCES**
 - in growth of children, 235
- RADIOIMMUNOASSAY**
 - conjugated bile salts, use in study of bile secretion, 202
 - serum somatomedins, 262
- RECOMMENDED DAILY ALLOWANCE**
 - niacin, discussion, SA289
 - vitamin E, SA161
- REHABILITATION**
 - of malnourished children, water and electrolyte changes, 74
- REQUIREMENTS**
 - histidine, for adults, 200
 - niacin
 - effect of dietary leucine on, review, SA289
 - in man (Nutrition Classic), 303
 - vitamin B₁₂, for man, SA65
 - vitamin D, special report, 61
- RETICULOENDOTHELIAL CELLS**
 - role in inflammation anemia, 213
- RETINOL**
 - metabolite, in urine, 244
- RIBOFLAVIN**
 - blood levels, in maternal and newborn, 298
 - deficiency, central nervous system abnormalities in, 21
 - losses, in food processing, 123
- RIBONUCLEIC ACID**
 - changes in brain, in vitamin B₆ deficiency, 21
- RICKETS**
 - experimental (Nutrition Classic), 48
 - hazards in overuse of vitamin D, special report, 61
- SALIVA**
 - secretion, in protein deficiency, 178
- SCHILLING TEST**
 - reliability for diagnosis of pernicious anemia, SA33
- SCURVY**
 - etiology (Nutrition Classic), 176
- SEBRELL, WILLIAM HENRY**
 - memorabilia on conquest of pellagra, 310
- SELENIUM** (see TRACE ELEMENTS)
- SELENOMETHIONINE**
 - availability of selenium in, 138
- SERUM**
 - ferritin levels, relation to body iron stores, 11
 - folate binder in, 9
- SEX DIFFERENCES**
 - in serum ferritin levels, 11
- SIALIC ACID**
 - metabolism, in alpha-1-antitrypsin deficiency disease, N189
- SICKLE CELL DISEASE**
 - zinc deficiency in, 266
- SILICON** (see TRACE ELEMENTS)
- SKELETON**
 - development, role of silicon in, SA257
- SKIN**
 - acrodermatitis enteropathica, 327
 - changes, in histidine deficiency, 200
 - copper in, after feeding excessive copper, 51
 - disease, in malnourished children, 334
 - potassium losses from, in malnourished children, 74
 - silicon content, SA257
- SKINFOLD THICKNESS**
 - in vegetarians versus controls, N285
 - measurement of body composition in infants, 7
- SMOKING**
 - nitrosamines in tobacco smoke, 19
- SOCIOECONOMIC DIFFERENCES**
 - in growth of children, 235
- SODIUM**
 - handling, by preterm infants, 105
- SOLANUM MALACOXYLON**
 - toxicity and vitamin D-like substances in, SA1
- SOLUBILITY**
 - zinc, in leavened and unleavened bread, 18
- SOMATOMEDINS**
 - relation to growth, 262
- SOUTHERN TUNISIA**
 - height-weight studies of children in, 235
- SPECIAL ARTICLES**
 - Bieri, J.G., Vitamin E, 161
 - Brown, A.T., The Role of Dietary Carbohydrates in Plaque Formation and Oral Disease, 353
 - Carlisle, E.M., Silicon, 257
 - Darby, W.J., Nutrition, Food Needs and Technologic Priorities: The World Food Conference, 225

- Darby, W.J., McNutt, K.W. and Todhunter E.N., Niacin, 289
- Halsted, C.H., The Small Intestine in Vitamin B₁₂ and Folate Deficiency, 33
- McCormick, D.B., Biotin, 97
- Mendeloff, A.I., Dietary Fiber, 321
- Mertz, M., Effects and Metabolism of Glucose Tolerance, 129
- Underwood, E.J., Cobalt, 65
- Wasserman, R.H., Active Vitamin D-Like Substances in Solanum malacoxylon and other Calcinogenic Plants, 1
- West, K.M., Prevention and Therapy of Diabetes Mellitus, 193
- SPECIAL REPORTS**
- An accessible archive of human experience, 310
- Effects of food processing on nutritional values, 123
- Exchange of personal nutrition collections, 312
- FAO/WHO handbook on human nutritional requirements, 1974, 147
- FTC proposed regulations on food advertising, 246
- Hazards of overuse of vitamin D, 61
- SPECIES VARIATION**
- in brain development, 6
- SPLEEN**
- incorporation of dietary selenium in, 138
- STARCH (see CARBOHYDRATE)**
- STARVATION**
- world crisis, World Food Conference, SA225
- STEELY-HAIR SYNDROME**
- copper metabolism in, N189
- STEROLS**
- fecal excretion, relation to diet, 136
- STOMACH**
- emptying and secretion, effect of dietary fiber on, SA321
- emptying time, dependence on caloric content of meal, 169
- lipase in, source of, 205
- STORAGE**
- iron, relation to serum ferritin levels, 11
- vitamin E, in various tissues, SA161
- SUCCINYL CoA**
- levels in liver, in vitamin B₁₂ deficiency, 85
- SUCROSE (see CARBOHYDRATE)**
- SULFATE**
- deficiency, retinal degeneration in, 343
- SULFATION**
- role of somatomedins in, 262
- SULFUR DIOXIDE**
- toxicity, in molybdenum deficiency, 185
- SURGERY**
- intestinal bypass, liver changes after, 38, 78
- SYNTHESIS**
- cholesterol, biliary, effect of taurocholate on, 202
- DNA, in HeLa cells, effect of folic acid binding protein on, 183
- elastin, role of copper in, 306
- fat, effect of hydroxycitrate on, 53
- fatty acids, role of biotin in, SA97
- gamma-aminobutyric acid, in brain, in vitamin B₆ deficiency, 21
- glucose, from alanine, defect in small-for-gestational-age infants, 168
- heme, in vitamin E deficiency, SA161
- lecithin, in liver, role of bile salts in, 202
- lipoproteins, in cellular insulin resistance, 199
- methionine, in folate deficiency, 118
- phospholipid, biliary, effect of taurocholate on, 202
- polyglutamates, in vitamin B₁₂ deficiency, 118
- polysaccharides, by dental plaque bacteria, SA353
- protein, in vitamin E synthesis, SA161
- taurine, in vitamin B₆ deficiency, 343
- thyroid hormone, role of cobalt, SA65
- xanthine oxidase, in vitamin E deficiency, 59
- TAURINE**
- in retinal and heart function, 343
- TAUROCHOLATE**
- effect on biliary lipid synthesis, 202
- TEETH**
- caries
- relation to dietary carbohydrate and dental plaque formation, SA353
- relation to salivary secretion of protein, 178
- eruption, in children, correlation with serum somatomedin levels, 262
- incorporation of selenium in, 138
- THIAMINE**
- blood levels, in maternal and newborn, 298
- deficiency
- central nervous system abnormalities in, 21
- pyruvate metabolism in (Nutrition Classic), 211
- losses, in food processing, 123
- THYROID (see also HORMONES)**
- changes
- in adult protein-calorie malnutrition, 299
- in goiter (Nutrition Classic), 272, 338
- endemic goiter, due to antithyroid agents, 171
- function, in undernutrition, 88
- metabolism, role of cobalt in, SA65
- thyroidectomy of fetus, cellular development after, 91
- TOBACCO SMOKE**
- nitrosamines in, 19
- TONGUE**
- lipase activity in, 205
- TONSIL**
- size, in malnourished children, 334
- TOTAL PARENTERAL NUTRITION**
- for low birthweight infants, 270
- TOXICITY**
- bisulfite, in molybdenum deficiency, 185
- botulinum and related toxins, 120
- chromium complexes, SA129
- cobalt, SA65
- copper, relation to tissue storage from, 51
- fluoride, genu valgum, 76
- macromolecules, in dental plaques, SA353
- niacin, review, SA289
- oxygen, protection by vitamin E, SA161
- phenylpyruvate, in experimental phenylketonuria, 276
- S. malacoxylon and other related plants, SA1
- vitamin D, special report, 61
- vitamin E, 269
- TRACE ELEMENTS**
- chromium, active ingredient of glucose tolerance factor, SA129
- cobalt, review, SA65
- copper
- deficiency, brain and heart changes in, 306
- metabolism in steely-hair syndrome, N189
- possible relation to osteosclerosis in fluorosis, 76
- storage of ionic form in Wilson's disease, 51
- fluoride, toxicity, genu valgum, 76
- iodine

- antithyroid agents in relation to endemic goiter, 171
- in prevention of goiter (Nutrition Classic), 272
- relation to structure of the thyroid gland (Nutrition Classic), 338
- manganese, possible relation to osteosclerosis in fluorosis, 76
- molybdenum, deficiency, bisulfite toxicity in, 185
- requirements for man, from FAO/WHO handbook, 147
- selenium
 - dietary sources, 138
 - relation to vitamin E deficiency, SA161
- silicon, review, SA257
- zinc
 - absorption in acrodermatitis enteropathica, 327
 - availability in leavened and unleavened bread, 18
 - deficiency, in sickle cell disease, 266
- TRANSPORT
 - calcium, in ileum, 84
 - folate and methotrexate, in vitamin B₁₂ deficiency, 118
 - mechanisms, in glucoreceptor cells, effect of phlorhizin on, 23
- TRISSETUM FLAVESCENS
 - toxicity and vitamin D-like substances in, SA1
- TUBERCULOSIS
 - in malnourished children, 334
- TUNISIA
 - Southern, height-weight measurements of children, 235, N350
- UREA
 - blood levels, after jejuno-ileal bypass, 38
 - output, on low protein intake, 180
- UREMIA
 - hearing disability in, N287
- UTILIZATION
 - alanine, in small-for-gestational-age infants, 168
 - amino acids, by crocodiles, 281
 - triglyceride, effect of prolactin on, 341
 - zinc, in unleavened bread, 18
- VEGETABLES
 - cobalt in, SA65
- VEGETARIANS
 - plasma lipid and lipoprotein levels in, N285
 - sterol and bile acid excretion in, 136
- VIRUSES
 - cause of diarrhea in children, N286
 - cause of neuropathies in chronic dialysis patients, N287
 - viral hepatitis A, new diagnostic tests for, 331
- VITAMIN A
 - beta-carotene, loss from supplemental iron in foods, 46
 - in mother's milk, 42, N191
 - losses, in food processing, 123
 - retinol, metabolite in urine, 244
- VITAMIN B₁ (see THIAMINE)
- VITAMIN B₂ (see RIBOFLAVIN)
- VITAMIN B₆
 - blood levels, in maternal and newborn, 298
 - deficiency
 - central nervous system abnormalities in, 21
 - taurine metabolism in, 343
 - pyridoxine, feeding, effect on brain and liver
 - pyridoxal phosphate levels, 214
- VITAMIN B₁₂
 - blood levels, in maternal and newborn, 298
 - deficiency
 - effect on CoA intermediates in the liver, 85
 - folate metabolism in, 118
 - in bats, neurological damage in, 217
 - intestinal absorption in, SA33
 - requirements for man, SA65
 - supplementation, effect on anemia in pregnancy, 332
- VITAMIN D
 - dihydroxycholecalciferol
 - similarity to S. malacoxylon factor, SA1
 - stereochemical orientation of, N29
 - hazards of overuse, special report, 61
 - hydroxycholecalciferol, therapeutic use, 209, N221
 - like substances in S. malacoxylon and other plants, SA1
 - promotes calcium deposition (Nutrition Classic), 48
- VITAMIN DEFICIENCY
 - etiology (Nutrition Classic), 176
- VITAMIN E
 - analogues, biological activity of, SA161
 - deficiency, xanthine oxidase in, 59
 - excess, effect on blood coagulability, 269
 - review, SA161
 - therapy, in premature babies, 206
 - α-tocopherol polyethylene glycol succinate,
 - absorption of, 206
 - tocopherols, loss from supplemental iron in foods, 46
- VITAMIN K
 - activity, in hypervitaminosis E, 269
 - role in carboxylation of glutamyl residues to produce prothrombin, 25
- VITAMINS
 - products, FDA proposals for regulation of, N220
- WATER
 - antithyroid agents in, 171
 - in feces, relation to dietary fiber, SA321
 - interstitial, in infants, measurement by skinfold thickness, 7
- WEIGHT
 - body
 - effect of undernutrition in newborn rats, 88
 - excessive, nephrosis in, 40
 - gain, of low birthweight infants on total parenteral nutrition, 270
 - measurements of children in Southern Tunisia, 235, N350
 - of rats, effect of hydroxycitrate on, 53
- WHEAT
 - bran, effect on colonic function, 70
 - wholemeal, phytates in, effect on zinc availability, 18
- WILSON'S DISEASE
 - ionic copper tissue storage in, 51
- WORLD FOOD CONFERENCE
 - SA225
- XANTHOMA
 - in type III hyperlipoproteinemia, 173
- YEAST
 - destruction of phytates by, in leavening bread, 18
 - Torula diet, effect on glucose tolerance, SA129
- ZINC (see TRACE ELEMENTS)



Nutrition Reviews

VOLUME 33

NUMBERS 1-12

January—December 1975

Published by

THE NUTRITION FOUNDATION, INC.

489 FIFTH AVENUE NEW YORK, N.Y. 10017

Copyright, 1975, Nutrition Foundation, Inc., New York, N.Y., U.S.A.

Editor

D. M. Hegsted, Ph.D., Professor of Nutrition
Schools of Medicine and Public Health
Harvard University, Boston

Associate Editor

Elmer H. Stötzt, Ph.D.
University of Rochester

Assistant Editor

Robin Holab

Production Manager

Marilyn K. Pliska

Contributing Editors

G. A. O. Alleyne, M.D. University of the West Indies	Robert B. McGandy, M.D. Harvard School of Public Health
Eric G. Ball, Ph.D. Marine Biological Laboratory, Woods Hole	Olaf Mickelsen, Ph.D. Michigan State University
Mahtab S. Bamji, Ph.D. National Institute of Nutrition, India	R. D. G. Milner, M.D., Ph.D. University of Manchester
G. E. Bunce, Ph.D. Virginia Polytechnic Institute and State University	Robert A. Neal, Ph.D. Vanderbilt University
Halvor N. Christensen, Ph.D. University of Michigan	Ralph A. Nelson, M.D., Ph.D. The Mayo Clinic
Richard M. Forbes, Ph.D. University of Illinois	Mildred S. Rodriguez, Ph.D. Los Angeles, California
Charles H. Halsted, M.D. University of California	James I. Shaw, Ph.D. Harvard School of Dental Medicine
Frank L. Iber, M.D. University of Maryland	D. A. T. Southgate, Ph.D. University of Cambridge
Kamala S. Jaya Rao, M.D. National Institute of Nutrition, India	Steven R. Tannenbaum, Ph.D. Massachusetts Institute of Technology
Michael C. Latham, M.B. Cornell University	Carol I. Waslien, Ph.D. Auburn University
Gilbert A. Leveille, Ph.D. Michigan State University	Mary Ann Williams, Ph.D. University of California
Richard L. Lyman, Ph.D. University of California	Benjamin J. Wilson, Ph.D. Vanderbilt University
Donald B. McCormick, Ph.D. Cornell University	Myron Winick, M.D. Columbia University

INFORMATION FOR SUBSCRIBERS

Subscriptions should be sent to the Nutrition Foundation, Inc., 888 Seventeenth St., N.W., Washington, D.C. 20006. Rate \$12.00 per year in the United States and other countries. A student rate of \$6.00 for the year 1976 is offered to regularly matriculated students in colleges or universities whose subscription application is accompanied by an affirmation of student status signed by an authorized Department representative. Changes of address should be sent to The Nutrition Foundation. In the U.S., ZIP CODES must be included.

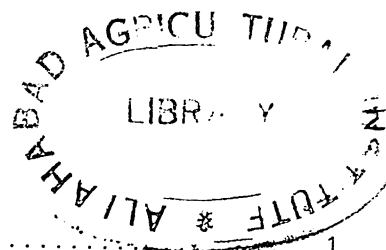
Note: Items may be quoted from NUTRITION REVIEWS without special permission. If quotations are made, the editors ask only that credit be given to NUTRITION REVIEWS and that they be made in such a way that the original meaning of the review is preserved.

The publication of NUTRITION REVIEWS has been undertaken to enable professionally trained people to keep abreast of current progress, and to have available an unbiased, authoritative review of the current research literature in the science of nutrition.

The Editorial Staff has been carefully selected so that the publication will merit, in reasonable degree, the confidence of the medical profession and others who work directly with the public in the field of nutrition. The service provided by the publication is distinct from that of an abstract journal, a review for laymen, or an extensive scientific review. Since the reviews as originally prepared by a member of the Editorial Staff are frequently modified by special referees and may be further modified by the Editorial Office in preparing final copy for the press, the Editorial Committee feels that it is preferable not to affix the names of Editorial Staff members to individual reviews. There is thus a high degree of group responsibility on the part of the Editor and Associate Editors.

Signed articles do not necessarily represent the opinion of the Editorial Staff.

CONTENTS OF VOLUME 33



NO. 1, JANUARY 1975

Active Vitamin D-Like Substances in Solanum malacoxylon and Other Calcinogenic Plants, by R.H. Wasserman	1
Growth of the Human Brain	6
Infant Body Composition by Skinfold Measurements	7
Folate Binder in Leukocytes and Serum	9
Serum Ferritin and Body Iron Stores	11
Edith G. Willcock and F. Gowland Hopkins: "The Importance of Individual Amino-Acids in Metabolism"	15
Zinc Availability in Leavened and Unleavened Bread	18
Nitrosamines and Cancer	19
Changes in Deficiency of Vitamin B ₆ and Other B-Complex Vitamins	21
Competition Between Phlorizin and Gold Thioglucose in Glucoreceptor Cells	23
Vitamin K and the Carboxylation of Glutamyl Residues	25
Termination of AMA Council	29
Meeting Announcements	30
FTC Proposal on Food Advertising	30
Letter to the Editor	31
Recent Books	31

NO. 2, FEBRUARY 1975

The Small Intestine in Vitamin B ₁₂ and Folate Deficiency, by C.H. Halsted	33
Obesity, Jejuno-Ileal Bypass and Death	38
Massive Obesity and Nephrosis	40
Lactation and Composition of Milk in Undernourished Women	42
Sucrose, Starch and Hyperlipidemia	44
Problems in Iron Enrichment and Fortification of Foods	46
E.V. McCollum, Nina Simmonds and J. Ernestine Becker: "Studies on Experimental Rickets"	48
Copper Toxicity, Rats and Wilson's Disease	51
Effect of (-)-Hydroxycitrate on Lipogenesis, Appetite and Body Weight in Rats	53
Hyperlipemia and Iron Deficiency	55
Hormonal Regulation of Formiminotransferase	57
Elevated Xanthine Oxidase in Vitamin E Deficiency	59
Hazards of Overuse of Vitamin D	61
Meeting Announcement	63
Current Status of AMA Council on Foods and Nutrition	64

NO. 3, MARCH 1975

Cobalt, by E. J. Underwood	65
Dietary Fiber and Colonic Function—An Effect of Particle Size?	70
Effects of Diet on Biliary Lipid Secretion and Bile Composition	72
Water and Electrolytes in Malnutrition	74
Genu Valgum Due to Fluoride Toxicity	76
Intestinal Bypass Surgery for Obesity	78
Archibald E. Garrod: "The Incidence of Alkaptonuria: A Study in Chemical Individuality"	81

Calcium Transport in the Ileum	84
Effect of Vitamin B ₁₂ Deprivation on CoA Intermediates Related to Propionate Metabolism	85
Thyroid Function in Experimental and Clinical Undernutrition	88
Cellular Development After Fetal Thyroidectomy	91
Recent Books	95
Meeting Announcements	95

NO. 4, APRIL 1975

Biotin, by Donald B. McCormick	97
The Relationship Between Infection and the Iron Status of an Individual	103
Renal Handling of Salt by Preterm Infants	105
Brain Growth in Kwashiorkor	107
Lymphocyte Function in Malnutrition	110
William Prout: "On the Ultimate Composition of Simple Alimentary Substances; with Some Preliminary Remarks on the Analysis of Organized Bodies in General"	112
Length of Poly-γ-Glutamyl Chains in Natural Folate	115
Folic Acid Metabolism in Vitamin B ₁₂ Deficiency	118
Interconversion of Bacterial Agents Causing Botulism and Gas Gangrene	120
The Effects of Food Processing on Nutritional Values	123
Fellowships in Clinical Nutrition	127
"Conquest of Famine" Receives Nutrition Foundations' Book Award	127

NO. 5, MAY 1975

Effects and Metabolism of Glucose Tolerance Factor, by Walter Mertz	129
Diet, Intestinal Flora and Colon Cancer	136
Studies on Selenium	138
Otto Folin: "A Theory of Protein Metabolism"	141
Iron and the Regulation of Erythropoiesis	144
FAO/WHO Handbook on Human Nutritional Requirements, 1974	147
New Fellowships in Nutrition Available for Physicians	158
Meeting Announcements	160
Recent Books	160

NO. 6, JUNE 1975

Vitamin E, by J. G. Bieri	161
Blunted Response to Oral Alanine Feeding	168
Gastric Emptying, Pancreatic and Biliary Secretion During Digestion	169
Endemic Goiter and Antithyroid Agents	171
Type III Hyperlipoproteinemia	173
Casimir Funk: "The Etiology of the Deficiency Diseases"	176
Malnutrition, Salivary Volume and Protein Concentration	178
Adaptation to Low Protein Intakes	180
The Inhibition of Cellular Uptake of Folate	183
Bisulfite Toxicity in Molybdenum-Deficient Rats	185
Effects of Oral and Parenteral Feeding on Pancreatic Enzyme Content	187
The Steely-Hair Syndrome	189
Alpha-1-Antitrypsin Deficiency—Liver Disease	189
Recent Publication	190
Letter to the Editor	191
Recent Books	191

NO. 7, JULY 1975

Prevention and Therapy of Diabetes Mellitus, by Kelly M. West	193
Insulin in Hypertriglyceridemia	199
Histidine: An EAA for Adults	200
The Secretion of Bile	202
Pharyngeal Lipase Activity in Man	205
Vitamin E Therapy in Premature Babies	206
Clinical Correlates of Vitamin D Metabolites and Calcium Metabolism	209
Rudolph A. Peters and Robert H.S. Thompson: "Pyruvic Acid as an Intermediary Metabolite in the Brain Tissue of Avitaminous and Normal Pigeons"	211
Anemia of Inflammation and Reticuloendothelial Cells	213
Liver Metabolism of Pyridoxal Phosphate	214
Neurological Damage in Vitamin B ₁₂ -Depleted Bats	217
FDA Proposals	220
The National Advisory Committee on Hyperkinesis and Food Additives	220
Anticonvulsant Drugs and Calcium Metabolism	221
Meeting Announcement	223

NO. 8, AUGUST 1975

Nutrition, Food Needs and Technologic Priorities: The World Food Conference, by William J. Darby	225
Heights and Weights of Children in Southern Tunisia	235
The Influence of Dietary Fat on the Composition of the Body Fat of Infants	236
Absorption of Extrinsic and Intrinsic Iron Labels	238
C. M. McCay, L. A. Maynard, G. Sperling and Leroy L. Barnes: "Retarded Growth, Life Span, Ultimate Body Size and Age Changes in the Albino Rat After Feeding Diets Restricted in Calories"	241
Protein-Bound Retinol or a Retinol Metabolite in Urine	244
FTC Proposed Regulations on Food Advertising	246
Recent Awards in Nutrition	255
New FAO Periodical	255
Recent Books	256
Meeting Announcements	256

NO. 9, SEPTEMBER 1975

Silicon, by Edith M. Carlisle	257
Somatomedins and Growth	262
Zinc Deficiency in Sickle Cell Disease	266
Cell-Mediated Immunity to Gliadin in Celiac Disease	267
Hypervitaminosis E and Coagulation	269
Oral Feeding Versus TPN in Low Birthweight Infants	270
David Marine and O. P. Kimball: "The Prevention of Simple Goiter in Man"	272
Urinary Phenylalanine Metabolites in Rats Treated with p-Chlorophenylalanine and Phenylalanine	276
Growth and Cerebral Lipid Composition in Rats Fed Linoleate and Linolenate	278
Persistence of Administered Amino Acids in Crocodilians	281
Bile Acid Excretion	285
Plasma Lipids and Lipoproteins in Vegetarians	285
Severe Diarrhea in Children	286
Neuropathy in Renal Patients on Chronic Dialysis	287

Fellowships in Clinical Nutrition	288
Announcement of Meetings	288

NO. 10, OCTOBER 1975

Niacin, by William J. Darby, Kristen W. McNutt and E. Neige Todhunter	289
Vitamin Levels at Term and in the Neonate	298
The Endocrinology of Adult Protein-Calorie Malnutrition	299
G. A. Goldsmith, H. L. Rosenthal, J. Gibbens and W. G. Unglaub: "Studies of Niacin Requirement in Man"	303
Brain and Myocardial Lesions in Copper-Deficient Young Rats	306
An Accessible Archive of Human Experience	310
Exchange of Personal Nutrition Collections	312
Grace A. Goldsmith, M.D.	314
Leaders in American Medicine	316
Committee on Nutritional Anthropology	318
Meeting Announcement	318
New NAS/NRC Report on Program Planning	319
Recent Books	319
D-Xylose Approved as a Diagnostic Agent	320

NO. 11, NOVEMBER 1975

Dietary Fiber, by Albert I. Mendeloff	321
Acrodermatitis Enteropathica—Hereditary Zinc Deficiency	327
EFA Deficiency in Continuous-Drip Alimentation	329
New Diagnostic Tests for Viral Hepatitis A	331
Iron Supplementation for Gestational Anemia	332
Immune Deficiency in Malnutrition	333
D. Marine and W. W. Williams: "The Relation of Iodin to the Structure of the Thyroid Gland"	333
The Effect of Prolactin on Lipoprotein Lipase Activity	341
Taurine Involvement in Retinal and Heart Muscle Function	343
Bihormonal Control of Ketogenesis	347
Letter to the Editor	349
Recent Books	351
FDA Toxicology Advisory Committee Formed	351
Meeting Announcement	351

NO. 12, DECEMBER 1975

The Role of Dietary Carbohydrates in Plaque Formation and Oral Disease, by Albert T. Brown	353
Author Index	365
Subject Index	377

The Nutrition Foundation, Inc.

Officers

J. George Harrar
Chairman
Board of Trustees

J. E. Lonning
Vice Chairman
Board of Trustees

William J. Darby
President

Clinton O. Chichester
Vice President

Richard M. Stalvey
Vice President

Trustees

Robert A. Aherne
President and General Manager
Finn-Cal Sweetener Company

J. Paul Austin
Chairman of the Board
The Coca-Cola Co.

Robert H. Autenrieth
Vice Pres.-Fresh Fruit Marketing
Sunkist Growers, Inc.

W. Gardner Barker
Chief Executive Officer and
Chairman of the Board
Thomas J. Lipton, Inc.

William O. Beers
Chairman of the Board and
Chief Executive Officer
Kraftco Corporation

Robert M. Bolz
President
Car Mayer & Company

John Z. Bowers
President
Isiah Macy Jr. Foundation

John B. Buckley
Group Vice President
Miles Laboratories, Inc.

John J. Burns
Vice President for Research
Hoffmann-LaRoche, Inc.

R. Craig Campbell
President
Welch Foods, Inc.

Herbert E. Carter
University of Arizona

William O. Coleman
Vice President
The Procter & Gamble Co.

Hugh E. Cumming
Senior Executive Vice President
Curtice-Burns, Inc.

William J. Darby
President
The Nutrition Foundation, Inc.

Val B. Diehl
President & Chief Operating Officer
Nabisco, Inc.

William J. Downey
Vice President and
Chief Flavor Chemist
Fritzsche-D & O, Inc.

Angier Biddle Duke
Commissioner of Public Events
New York, New York

B. L. Durling
President
Stange Company

James W. Evans
President
American Maize-Products Co.

Robert T. Foote
President
Universal Foods Corporation

Clarence Francis
New York, New York

Jack N. Friedman
President
Florasynth, Inc.

Robert L. Gibson, Jr.
President
California Cannery & Growers

Samuel Goldblith
Massachusetts Institute of
Technology

James D. Grant
Vice President
CPC International Inc.

F. W. Griffith
President
Griffith Laboratories, Inc.

David E. Guerrant
President
The Nestle Company, Inc.

Phillip Handler
President
National Academy of Sciences

J. George Harrar
New York, New York

Henry J. Heinz, II
Chairman of the Board
H. J. Heinz Company

Victor W. Henningsen, Jr.
President
Henningsen Foods, Inc.

David S. Hollingsworth
General Manager
Hercules, Inc.

E. F. Huskisson
Group Vice President
Swift & Company

George W. Irving, Jr.
FASEB

David P. Jaicks
President
American Home Foods, Division of
American Home Products

Jullus E. Johnson
Vice President
The Dow Chemical Company

Morley R. Kare
Director
Monell Chemical Senses Center

C. Glen King
Kennett Square, Pennsylvania

E. Robert Kinney
President and Chief
Operating Officer
General Mills, Inc.

R. D. Kummel
Senior Vice President
Carnation Company

Richard Laster
Group Vice President
General Foods Corporation

Vacancy
Libby, McNeill & Libby

Dale R. Lindsay
National Center for
Toxicological Research

Herbert E. Longenecker
New Orleans, Louisiana

J. E. Lonning
Chairman & Chief Executive Officer
Kellogg Company

Luke A. Marano
President
Philadelphia Macaroni Co., Inc.

A. R. Marusi
Chairman & President
Borden, Inc.

Kenneth Mason
Executive Vice President
The Quaker Oats Company

William McElroy
Chancellor
University of California, San Diego

C. Dean McNeal
Group Vice President
The Pillsbury Company

H. B. Morley
Chief Executive Officer
Stauffer Chemical Company

Emil Mrak
Chancellor Emeritus
University of California, Davis

Thomas C. Munnell
Vice President, Admin.
William Underwood Company

J. Quigg Newton
Vice Chairman of the Board
The Commonwealth Fund

George M. Perry
President
Dobbs-Life Savers, Inc.

Edward J. Piszek
President
Mrs. Paul's Kitchens, Inc.

Gretchen Pullen
Swanson Foundation

Robert H. Pulver
Executive Vice President
H. Kohnstamm & Company, Inc.

Robert T. Quilmeyer
President
Amstar Corporation

Robert M. Rapaport
President & Chief Operating Officer
SuCrest Corporation

Wilson Riles
Superintendent of Public Instruction
and Director of Education
State of California

Boyd F. Schenk
President & Chief Executive Officer
Pet Incorporated

Frederick Seitz
President
Rockefeller University

Harold A. Shaulb
President
Campbell Soup Company

Reginald A. Sinclair
Senior Vice President
Royal Crown Cola Company

Horace L. Sipple
Naples, Florida

Lloyd E. Skinner
Chairman of the Board
Skinner Macaroni Company

Louis C. Smith, Jr.
Vice President, Technical
Hershey Foods Corporation

John C. Suerth
Chairman of the Board and
Chief Executive Officer
Gerber Products Company

Luther L. Terry
University Associates, Inc.

Kenneth E. Wattman
Group Vice President
Specialty Chemicals Group
ICI United States Inc.

Henry Weigl
President
Standard Brands, Inc.

Harry K. Wells
President
McCormick & Company, Inc.

Ross B. Yerby
Vice Chairman of the Board
Del Monte Corporation

* Representative of the Public

MEMBERS OF THE FOUNDATION

American Home Foods
American Maize-Products Corp.
Amstar Corporation
Borden, Inc.
California Canners & Growers
Campbell Soup Company
Carnation Company
Coca-Cola Company
CPC International, Inc.
Curtice-Burns, Inc.,
Del Monte Corporation
Dobbs-Life Savers, Inc.
Dow Chemical Company
Esmark, Inc.
Finn-Cal Sweetener Company
Florasynth Labs, Inc.
Fritzsche-D&O
General Foods Corporation

General Mills, Inc.
Gerber Products Company
Griffith Laboratories, Inc.
Heinz Company, H. J.
Henningsen Foods, Inc.
Hercules, Inc.
Hershey Foods Corporation
Hoffmann-LaRoche, Inc.
ICI United States Inc.
Kellogg Company
Kohnstamm, H., & Co., Inc.
Kraftco Corporation
Libby, McNeill & Libby
Lipton, Inc., Thomas J.
McCormick & Company, Inc.
Miles Laboratories, Inc.
Mrs. Paul's Kitchens, Inc.
Nabisco, Inc.

Nestle Company, Inc.
Oscar Mayer & Company
Pet Incorporated
Philadelphia Macaroni Company
Pillsbury Company
Procter & Gamble Company, Inc.
Quaker Oats Company
Royal Crown Cola Company
Skinner Macaroni Company
Standard Brands, Inc.
Stange Company
Stauffer Chemical Company
SuCrest Corporation
Sunkist Growers, Inc.
Underwood Company, William
Universal Foods Corporation
Welch Foods, Inc.

FOOD AND NUTRITION ADVISORY COUNCIL

The Food and Nutrition Advisory Council advises the Foundation with respect to research, training, education and other policy matters.

Howard E. Bauman
The Pillsbury Company

Earl E. Lockhart
The Coca-Cola Company

O. Neal Miller
Hoffmann-LaRoche, Inc.

Richard L. Hall
McCormick & Company, Inc.

Walter J. Mars
Arkansas State University

Arthur I. Morgan, Jr.
U. S. Department of Agriculture

D. Mark Hegsted
Harvard School of Public Health

Karl E. Mason
National Institutes of Health

Nevin S. Scrimshaw
Massachusetts Institute of Technology

FOOD AND NUTRITION LIAISON COMMITTEE

The Committee serves the Foundation by reviewing scientific areas with a view to identifying broad new research and educational interests. The committee also assists in evaluating research and educational proposals and with the evaluation of institutions with capabilities of conducting research projects. It also serves to relate the Foundation's programs to the scientific and technical community.

James J. Albrecht
John C. Bard
Howard E. Bauman
Dwight H. Bergquist
Samuel B. Blaskey
O. N. Breivik
W. A. Brittin
Charles B. Broeg
John J. Burns
Hugh R. Butt
J. Alexander Campbell
Lorne Campbell
Carlos Caridad
J. Hoyt Chaloud
Richard J. Coleman
Alfred S. Cummin
Ignacio Deschamps
Andrew G. Ebert

Robert J. Eiserle
Enio Feliciotti
L. J. Filer, Jr.
E. M. Foster
I. E. Friedman
E. C. Galloway
Samuel Goldblith
Willis A. Gortner
Richard A. Greenberg
William C. Griffin
Richard L. Hall
John A. Harpham
J. George Harrar
W. J. Hart, Jr.
Robert O. Herrmann
Richard E. Hine
John H. Hopper
Paul F. Hopper

Paul E. Johnson
Victor L. Johnson
Charles Johnston
C. H. Krieger
Gilbert A. Leveille
Hugh R. Linn
Earl E. Lockhart
John V. Luck
L. E. Lynch
Donald A. M. Mackay
Luke A. Marano
J. M. McIntire
O. Neal Miller
W. T. Miller
E. L. Mitchell
Roland A. Morck
Emil Mrak
R. O. Nesheim

Charles F. Niven
C. J. O'Donovan
Howard Pearson
Paul E. Ramstad
Dorothy M. Rathmann
Gerald Reed
Louis Sair
James Scala
Bernard S. Schweigert
C. Mickey Skinner
Charles A. Stevenson
Robert A. Stewart
Vern L. Stromberg
Philip L. White
Myron Winick
Barry L. Zoumas
Samuel Zuckerman

The Nutrition Foundation, Inc.

Officers

J. George Harrar
Chairman
Board of Trustees

J. E. Lonning
Vice Chairman
Board of Trustees

William J. Darby
President

Clinton O. Chichester
Vice President

Richard M. Stalvey
Vice President

Trustees

Morton Adams
President
Curtice-Burns, Inc.

Richard D. Arney
Executive Vice President
and Director
Esmark Inc.

E. L. Artzt
Vice Pres.-Group Executive
The Procter & Gamble Co.

J. Paul Austin
Chairman of the Board
The Coca-Cola Co.

Robert H. Autenrieth
Vice Pres.-Fresh Fruit Marketing
Sunkist Growers, Inc.

W. Gardner Barker
Chief Executive Officer and
Chairman of the Board
Thomas J. Lipton, Inc.

William O. Beers
Chairman of the Board and
President
Kraftco Corporation

Robert M. Bolz
President
Oscar Mayer & Company

***John Z. Bowers**
President
Josiah Macy Jr. Foundation

John B. Buckley
Group Vice President
Miles Laboratories, Inc.

John J. Burns
Vice President for Research
Hoffmann-LaRoche, Inc.

R. Craig Campbell
President
Welch Foods, Inc.

***Herbert E. Carler**
University of Arizona

***John Cooper**
Director
Association of American
Medical Colleges

***William J. Darby**
President
The Nutrition Foundation, Inc.

Val B. Diehl
President & Chief Operating Officer
Nabisco, Inc.

William J. Downey
Vice President and
Chief Flavor Chemist
Fritzsche-D & O, Inc.

***Angier Biddle Duke**
Commissioner of Public Events
New York, New York

B. L. Durling
President
Strange Company

James W. Evans
President
American Maize-Products Co.

Robert T. Foote
President
Universal Foods Corporation

***Clarence Francis**
New York, New York

Jack N. Friedman
President
Florasynth, Inc.

Vincent A. Fulmer
Vice President, Admin.
William Underwood Company

Robert L. Gibson, Jr.
President
California Cannery & Growers

***Samuel Goldblith**
Massachusetts Institute of
Technology

James D. Grant
Vice President
CPC International Inc.

F. W. Griffith
President
Griffith Laboratories, Inc.

David E. Guerrant
President
The Nestle Company, Inc.

***Phillip Handler**
President
National Academy of Sciences

***J. George Harrar**
New York, New York

Robert S. Hatfield
Chairman and President
Continental Can Company, Inc.

Henry J. Heinz, II
Chairman of the Board
H. J. Heinz Company

Victor W. Henningsen, Jr.
President
Henningsen Foods, Inc.

***Theodore M. Hesburgh, C.S.C.**
President
University of Notre Dame

***George W. Irving, Jr.**
FASEB

Julius E. Johnson
Vice President
The Dow Chemical Company

Ebon C. Jones
Executive Vice President
Owens-Illinois

***Morley R. Kare**
Director
Monell Chemical Senses Center

***C. Glen King**
Institute of Human Nutrition
Columbia University

E. Robert Kinney
President and Chief
Operating Officer
General Mills, Inc.

R. D. Kummel
Senior Vice President
Carnation Company

Richard Laster
Group Vice President
General Foods Corporation

***Dale R. Lindsay**
Duke University Medical Center

***Herbert E. Longenecker**
President
Tulane University

J. E. Lonning
Chairman & Chief Executive Officer
Kellogg Company

Luke A. Marano
President
Philadelphia Macaroni Co., Inc.

A. R. Marusi
Chairman & President
Borden, Inc.

Kenneth Mason
Group Vice President
The Quaker Oats Company

***William McElroy**
Chancellor
University of California, San Diego

C. Dean McNeal
Group Vice President
The Pillsbury Company

H. B. Morley
Chief Executive Officer
Stauffer Chemical Company

***Emil Mrak**
Chancellor Emeritus
University of California, Davis

***J. Quigg Newton**
President
The Commonwealth Fund

William O. Nicoll
General Manager
Hercules, Inc.

***Paul B. Pearson**
Universidad Autonoma de
Guadalajara

George M. Perry
President
Dobbs-Life Savers, Inc.

Edward J. Piszek
President
Mrs. Paul's Kitchens, Inc.

***Gretchen Pullen**
Swanson Foundation

Robert H. Pulver
Executive Vice President
H. Kohnstamm & Company, Inc.

Robert T. Quillmeyer
President
Amstar Corporation

Robert M. Rapaport
President & Chief Operating Officer
SuCrest Corporation

***Wilson Riles**
Superintendent of Public Instruction
and Director of Education
State of California

Boyd F. Schenk
President & Chief Executive Officer
Pet Incorporated

***Frederick Seltz**
President
Rockefeller University

Harold A. Shaub
President
Campbell Soup Company

Joseph B. Shortlidge
President
American Home Foods, Division of
American Home Products

Reginald A. Sinclair
Senior Vice President
Royal Crown Cola Company

***Horace L. Sipple**
Naples, Florida

Lloyd E. Skinner
Chairman of the Board
Skinner Macaroni Company

Louis C. Smith, Jr.
Vice President, Technical
Hershey Foods Corporation

John C. Suerth
Chairman of the Board and
Chief Executive Officer
Gerber Products Company

***Luther L. Terry**
University Associates, Inc.

Kenneth E. Wattman
Group Vice President
Specialty Chemicals Group
ICI United States Inc.

Henry Weigl
President
Standard Brands, Inc.

Harry K. Wells
President
McCormick & Company, Inc.

Ross B. Yerby
Vice Chairman of the Board
Del Monte Corporation

* Representative of the Public